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HANS J. TRURNIT

TECHNICAL PAPERS

COMMENTS AND COMMUNICATIONS

NEWS AND NOTES

CONFERENCES ON COSMIC RADIATION AND HIGH ALTITUDE BIOLOGY

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VOLUME 111, NUMBER 2871

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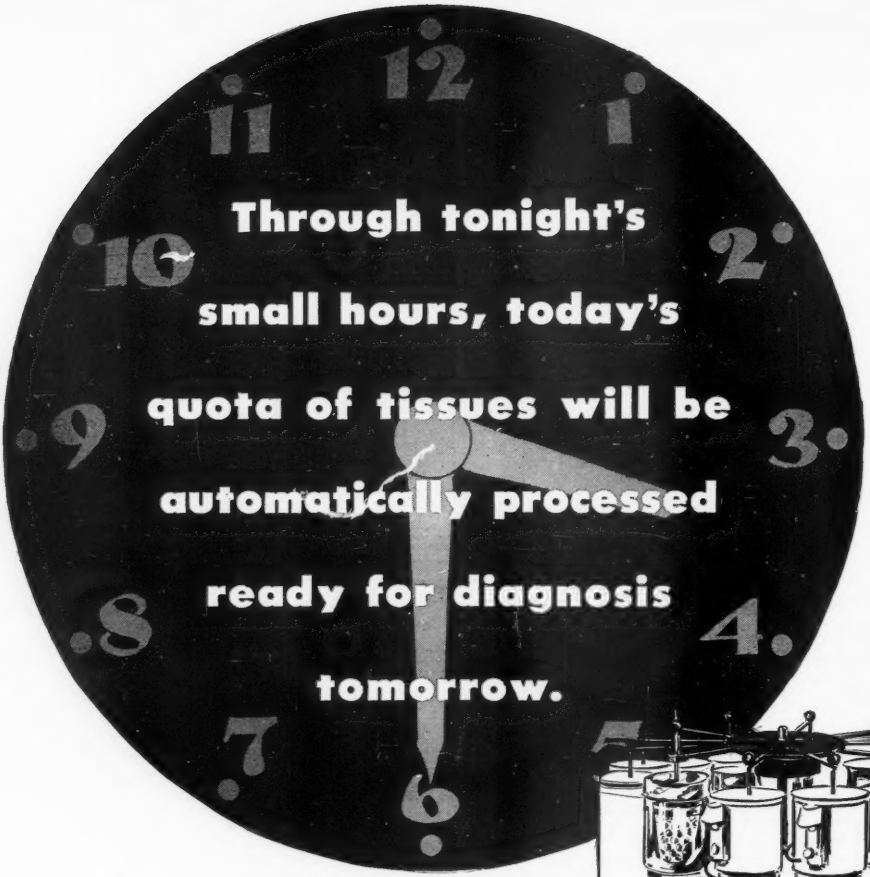

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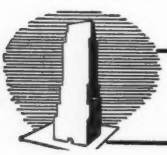

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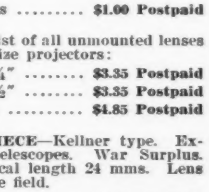

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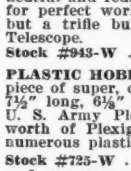

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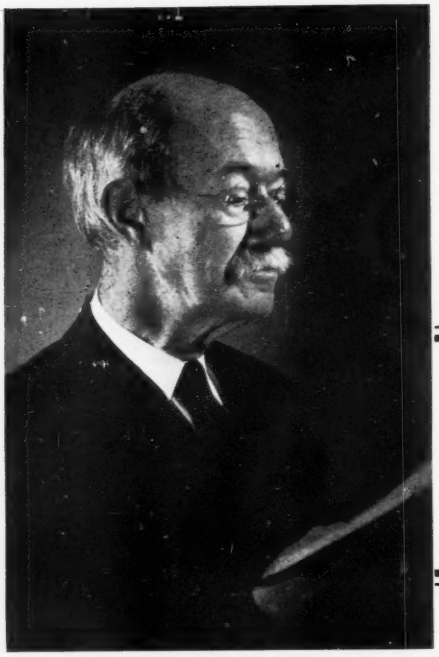

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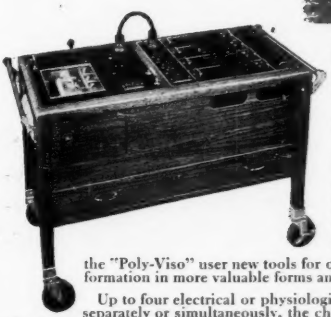

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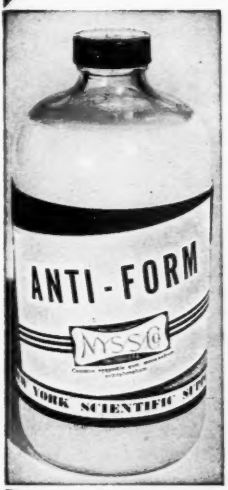

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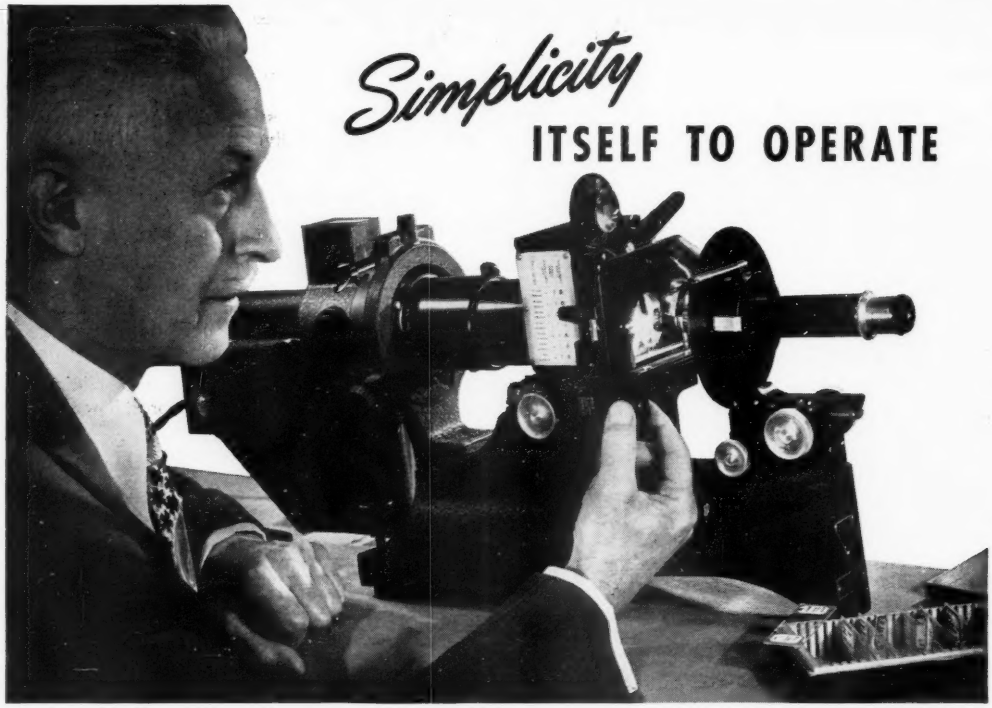

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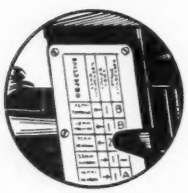

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Protein Permeability Through Thin Organic Layers[1]

Hans J. Trurnit

Medical Division, Army Chemical Center, Maryland

IT HAS BEEN KNOWN for several years (*1, 2, 14*) that specific adsorption of antibody molecules up to several hundred angstrom units thick takes place when a plate covered with molecular layers of antigenic protein is treated for a few minutes with diluted antiserum. With normal or heterologous serum only a very small nonspecific adsorption amounting to 10–20 A occurs.

In 1942, Rothen and Landsteiner (*14*) made the following observations:

> When, after transfer of an antigen film to a metal slide, a double layer of stearic acid-stearate was deposited on top of the antigenic protein, there was the normal increase of 48 A in thickness, but the plate came out wet instead of dry.[2] On treatment with a homologous antiserum unexpectedly a specific increase, 30 to 40 A, was observed; after covering with three double layers of stearic acid, instead of one as above, this effect no longer took place. Since a reaction between antigen and antibody, in spite of interposed layers, would seem improbable, one must consider that perhaps there were discontinuities or uncovered areas in the stearic acid film, or that a displacement had occurred which would bring the protein film above the stearic layer.

Since then Rothen has published a series of papers (*7–9–11*) on this interaction between protein molecules through screening films in which he abandoned the original explanation. His experimental evidence and considerations of related facts lead him to the postulate of long range forces between certain types of protein molecules. He states (*13*): "This long range interaction might take place through resonating extended oscillators, the presence of which is likely to occur in large molecules as suggested by London."

Considering the evidence for the necessity of close contact between specific groups of antigen and antibody molecules put forth by Pauling (*5, 6*) and others (*3, 4*) and considering the activation energy of many kilocalories necessary for the breakup of peptide bonds by the action of trypsin,[3] it is difficult to accept Rothen's long range forces explanation.

By following his basic experimental procedure, we have found it possible to reproduce all the main points of his published data. Certain deviations from Rothen's technique are listed below. The question to be resolved is not whether Rothen's experiments are correct but whether his proposed explanation is valid.

Assuming that there are no long range forces, but some kind of protein permeability of the screening material, we should expect better protection by multiple layers of screening material than by single layers of the same total thickness. By analogy, a set of sieves with random hole distribution placed tightly on top of each other is more impermeable than a single thick sieve with the same hole distribution. Our experimental results with multiple screens consisting of more than three layers of Formvar confirm strongly the validity of such an assumption, with the exception that a purely geometrical explanation is too simple.

With very thin single or multiple Formvar screens, the specific adsorption from antiserum is usually as high as, or even higher than, without screen. This is due to the fact that the leaching of antigenics material (see below) occurs faster than the formation of specific precipitate with the respective concentrations of buffer and antiserum used. The experimental evidence for this statement will be given elsewhere. Obviously, the presence of a very thin screen slows the leaching process without interfering too much with the specific reaction.

At another point in our investigation, we realized that no data had been presented by Rothen on the action of the saline phosphate buffer itself on a screen-covered antigen plate. Saline phosphate buffer was used for diluting the sera in all his experiments as well as ours. This control experiment was made and immediately suggested a simple explanation of the Rothen effect for the antigen-antibody system.

As shown in the experimental part, saline phosphate buffer or phosphate buffer removes antigen material from underneath the screen, apparently through holes.[4] If the plates are treated with distilled water, even for 40 minutes, there is no loss of antigenic material. Veronal buffer, as used in the trypsin experiments, removes antigenic material to a much smaller extent and only through very thin screens.

In experiments with saline phosphate buffer or phosphate buffer, antigen penetrated the screen and could be removed from the plate by washing with dis-

[1] Grateful acknowledgment is made of the generous assistance given by Dr. A. Rothen in this investigation.

[2] A metal plate covered only with several layers of stearic acid-stearate is water-shedding.

[3] The system trypsin-substrate has an activation energy of 10–15 kg-cal/mole, depending on the substrate.

[4] During a conversation on 12 September 1949, the author learned that Dr. Rothen had discovered independently the leaching effect of phosphate ions.

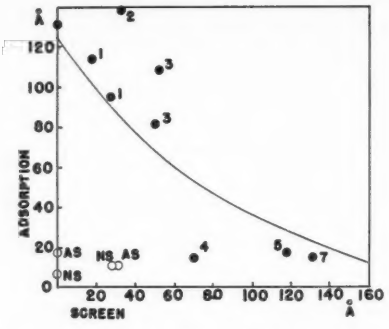

FIG. 1. Adsorption of rabbit antibody through Formvar screen on two double layers of serum albumin. Abscissas give total screen thickness. Inserted figures indicate the numbers of individual Formvar films which build up the screen. Each point represents the average of between four and eight individual experiments done under identical conditions. The curve is a reproduction of Rothen's curve for the same conditions. Our points, however, represent the true increment, i.e., we did not deduct a certain amount of nonspecific adsorption, as Rothen did in his curve. Depending on the screen thickness, 5–20 A has to be deducted from our points for comparison with Rothen's curve (see Fig. 4). *AS* and *NS* represent adsorption from antiserum and normal serum on plates without antigen.

tilled water. After drying the plate, the decrease in thickness was easily measurable with an ellipsometer.[5] The amount of decrease is a function of time. Treatment of the plate thereafter with antiserum yielded a correspondingly smaller specific adsorption. When the plate is directly treated with diluted antiserum instead of saline buffer, the antigen meets the antibody as soon as it comes to the screen surface, and forms a precipitate which cannot be removed from the plate by washing in the usual way. When the plate was pretreated with diluted normal serum instead of saline buffer, the leaching effect was much stronger in case of very thin screens or on plates without screen. For thicker screens, however, this difference did not exist. Nevertheless, the treated part of the plate showed a much smaller specific adsorption of antibody after a subsequent treatment with antiserum than the control part. Treatment with normal serum gives a combined effect of decrease by loss of antigenic material and increase by unspecific adsorption of serum proteins.

It is easily possible to demonstrate the antigen which has passed the screen by placing against the screen-covered antigen plate a normal conditioned plate with a droplet of saline phosphate buffer between the two plates. After 10 minutes an increase in thickness occurs on the second plate (pickup plate) after washing and drying. A subsequent treatment with antiserum shows a specific adsorption on the pickup plate. If this experiment is performed with diluted normal serum instead of saline phosphate buffer, there is no specific adsorption. Obviously, the antigen cannot be adsorbed under those conditions on the pickup plate.

With the enzyme system, trypsin-serum albumin, the behavior is somewhat more complicated because trypsin itself appears to be able to penetrate the screen.

In conclusion, we seem to be facing the problem of a differential protein permeability of thin organic layers rather than the long range forces postulated by Rothen. This preliminary report includes a description of deviations from Rothen's original technique and the high lights of experimental results.

Optical Base. In order to get several comparable figures at the same time under identical conditions, we did not use a single boundary line between one and three or three and five layers of barium stearate on each plate, but we used ten steps, which provided us with nine independent boundary lines for measurement by matching. To increase our sensitivity, these steps were built on a uniform base of 19.5 double layers of barium stearate so that the numbers of single layers for successive steps were: 39, 41, 43 . . . up to 57. The maximum sensitivity with such a plate is between 10 and 11 degrees of analyzer rotation for a 50-A increment in thickness. A calibration curve was made for each plate. The standard deviation of our readings (determined with sets of ten readings for each

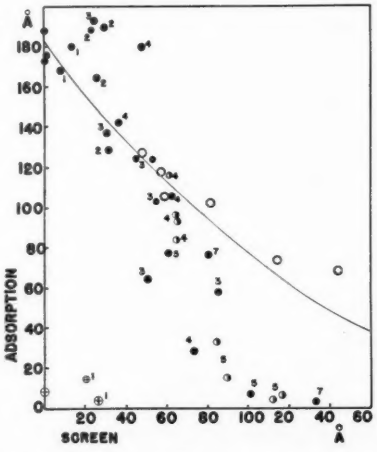

FIG. 2. Adsorption of rabbit antibody through Formvar screen on three double layers of serum albumin. Abscissas give total screen thickness. For the inserted figures and curve see the legend of Fig. 1. Open circles indicate experiments with single cast screens (Rothen's technique). Crossed circles indicate experiments on plates without antigen. Closed circles and half-closed circles represent experiments with multiple screens. The closed circles represent averages of between four and eight individual experiments, whereas the eight half-closed circles represent single readings on a plate half of whose surface was covered with four layers and the other half with five layers of Formvar.

[5] A term coined by A. Rothen for his polarization spectrometer (8).

step) was ± 0.5 A for the most sensitive part of the plate and ± 2.2 A for the least sensitive step. The experiments were made with an angle of incidence, $\varphi = 70°$. The plates were microscope slides coated with pure chromium. The optical base was always deposited on five plates simultaneously by means of a dipping device operated by oil pressure, which gave an extremely smooth and homogeneous linear movement in both directions.

Spreading of Antigen. To make sure that we did not have a loss of serum albumin into the substrate, we developed a new technique for spreading proteins which will be presented later. Usually there is an appreciable loss of protein into the substrate if a droplet of protein solution is placed on a water surface, even if this is done very carefully. This dissolved material may cause a film of undenatured protein molecules adsorbed underneath the spread protein film. For the problem under investigation it is important to avoid this error as much as possible. The thickness for three double layers of serum albumin spread by our method and deposited under a pressure of between 8 and 9 dynes per cm was always around 57 A.

Screen Casting. In case of thick single screens, we followed Rothen's original technique by dipping a clean glass slide into a solution of Formvar or collodion and depositing the dried film on a clean water surface, from which it was then transferred to the experimental plate. For thin screens we spread the solution of plastic material directly on a clean water surface and deposited it on the experimental plate by passing this plate nearly horizontally face down through the water surface, turning the plate upside down under water, cleaning the surface with a glass bar and bringing the plate up through the clean water surface. The multiple screens were all deposited in this way.

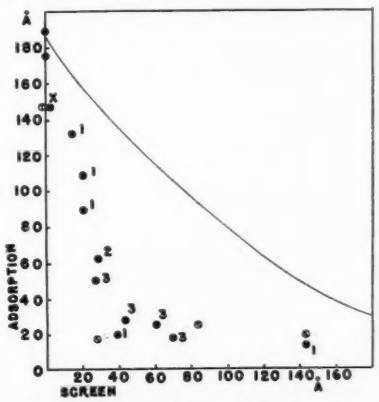

FIG. 3. Adsorption of rabbit antibody through collodion screen on three double layers of serum albumin. Abscissas give total screen thickness. Closed circles have the same meaning as in Figs. 1 and 2. Circles with oblique bar are experiments with normal serum. Circle with upright bar and the closed circle next to it (*X*) are two experiments without screen where one plate was dipped for a few seconds into pure amyl acetate and dried and then treated together with the control plate with antiserum, which in this case was a little older. This control was necessary to show that the deposition of collodion films from a substrate saturated with amyl acetate did not affect the antigenic properties of the serum albumin.

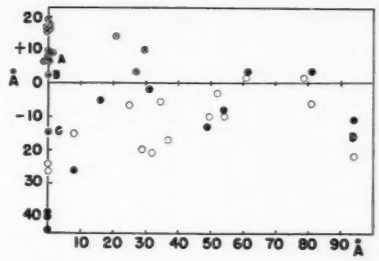

FIG. 4. Nonspecific adsorption from antiserum and action of saline phosphate buffer and normal serum. The ordinates give the increase or decrease of total thickness from plates with and without three double layers of serum albumin coated with multiple Formvar screens. The abscissas give the screen thickness. Crossed circles represent adsorption from diluted antiserum on plates without antigen. Open circles represent action of saline phosphate buffer on antigen plates. Closed circles represent action of diluted normal serum on antigen plates. Circles with black centers represent the action of diluted normal serum on plates without antigen (*A*), on plate with a single layer of antigen (*B*) and on plate with a single plus a double layer of antigen (*C*). *D* represents the action of saline phosphate buffer and diluted normal serum on antigen plate with single cast Formvar film.

Treatment with Sera. Instead of smearing a serum droplet over the slide, we deposited a serum droplet at the end of the slide and covered the plate with a microscope cover slide cut to the suitable size. The experimental area on our plates was 25 × 40 mm. The cover glasses were 22 × 50 mm. In covering the plate with this size cover glass we used 0.15 ml of diluted serum. In covering the plate with slides 9 × 40 mm, we used 0.05 ml of diluted serum. Thus the liquid film was in both cases about 135 μ thick. We believe this method of application gives more regular results. In all experiments we used a 10-minute treatment period.

Washing. We did not wash our plates after depositing the antigenic material, but we washed the plates once with saline buffer and twice with double-distilled water after treatment with sera.

Materials used. As antigen, we used crystallized bovine plasma albumin (Armour and Company) dissolved in double-distilled water. Normal serum was obtained from a healthy rabbit. The antiserum (rabbit) was kindly provided by Dr. M. Mayer of the Johns Hopkins School of Hygiene. It contained 0.96 mg antibody-nitrogen per ml. Formvar 15/95 "E" (from Shawinigan Products Corporation in New York City) was dissolved in redistilled dichloroethane,

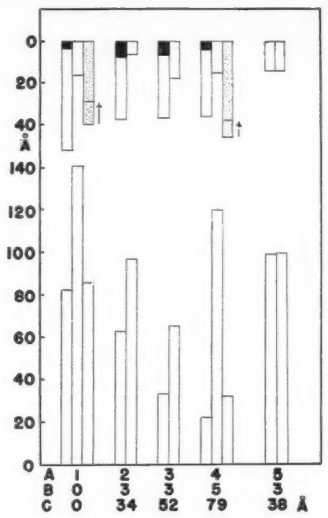

FIG. 5. Experiments with pickup plates. Ordinates represent adsorption. The diagram represents five experiments where a plate with three double layers of antigen (and multiple Formvar screen in experiments 2–5) has been covered by a conditioned plate with a droplet of saline phosphate buffer, veronal buffer, or diluted normal serum in between. A control strip was always left free. After washing and drying these plates, all plates were treated with diluted antiserum. The lower part of the diagram represents the adsorption of antibody on the five base plates after the pretreatment mentioned. The long column on each plate is always the adsorption on the control strip. The left column is the adsorption on the part which has been treated previously with saline buffer (experiments 1–4) or veronal buffer (experiment 5). In experiments 1 and 4 the right-hand column is the adsorption on the part which has been treated previously with diluted normal serum. *A* represents number of experiment, *B* represents number of single Formvar layers which build up the total screen, and *C* represents the total screen thickness in angstrom units.

The upper part of the diagram represents the findings on the pickup plates. The black sections show the actual pickup of antigen after the plates were in contact with the base plates. The dotted columns in experiments 1 and 4 show a nonspecific protein adsorption from normal serum probably mixed with antigen. The white sections and columns show the adsorption after treatment with diluted antiserum. On the two parts in experiments 1 and 4 which were treated previously with diluted normal serum, a loss of 5–10 A occurred after the antiserum treatment which is indicated by the two arrows.

Control experiments showed that a conditioned optical base does not adsorb more than the usual small amount of nonspecific material from antiserum after a 10-minute treatment with phosphate saline buffer.

and collodion U. S. P. (Merck) containing alcohol and ether was diluted by adding amyl acetate. Collodion films were spread on double-distilled water saturated with amyl acetate. Formvar films were spread on pure double-distilled water.

The first three figures represent part of our indirect evidence for the existence of a protein permeability through Formvar and collodion films. Other indirect evidence has already been presented by S. J. Singer (15). Figs. 4 and 5 represent the first direct evidence to this effect. The legends give all the necessary information.

TABLE 1

INFLUENCE OF TIME ON THE INTERACTION BETWEEN SALINE BUFFER AND A SCREEN-COVERED ANTIGEN PLATE

Time of pretreatment min	Increment after pretreatment A	Adsorption from antiserum A
0	0	142
1	−7	111
4	−20	83
8	−22	73
16	−24	70

The data in Table 1 were obtained with a plate having three double layers of serum albumin and a Formvar film of 37 A total thickness, consisting of four single Formvar layers, dipped with its longer edge horizontal to a depth four-fifths of its width into saline phosphate buffer and withdrawn by a fifth of its width at definite time intervals, so that the treatment time was 1, 4, 8 and 16 minutes. After washing with double-distilled water and drying, the plate was measured and then treated for 10 minutes with diluted antiserum as usual.

The work presented here in this paper was performed during the period April to August 1949.

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The work presented here in this paper was performed during the period April to August 1949.

Technical Papers

An Electronic Method of Tracing the Movements of Soil-inhabiting Insects[1]

A. P. Arnason,[2] R. A. Fuller,[3] and J. W. T. Spinks[4]

Dominion Entomological Laboratory, Saskatoon, and *Department of Chemistry, University of Saskatchewan, Saskatoon, Canada*

Recently a new method of following the movements of soil-inhabiting insects has been developed, using a radioactive tracer technique. This new technique has been applied so far only to the larva of the prairie grain wireworm, *Ctenicera aeripennis destructor* (Brown), but it is applicable to many species.

Up to the present, information on wireworm movements has come from observations using other than natural environmental conditions (*1, 5*). Much information on movements of the wireworm population as a whole in response to seasonal and other environmental changes has been gathered by actually removing the larvae from their natural habitat and noting their position at time of removal (*2, 3, 4*). This has the disadvantage that the activity of individuals or groups of individuals cannot be followed. The use of a radioactive tracer seems to offer a method of studying wireworm behavior under approximately normal circumstances (*6*).

This tracer method involves making the larva radioactive in some way so that its movements may be traced, from above the soil, by means of a Geiger-Müller tube. The feeding of activity to the larva as a means of activation is not practical for several reasons, whereas attaching activity to the larva shows some degree of usefulness. However, external application is suitable only between molts, because the activity is shed at each ecdysis. Investigations to date have indicated that the most efficient means of activating the larva will probably be by the insertion of radioactive cobalt wire into the body cavity of the wireworm. Successful insertions, using inactive wire, have already been carried out, with a high survival rate of treated larvae for both wireworms and cutworms. No apparent abnormality or loss of movement was evident in the surviving wireworm larvae up to three months after treatment. The treated cutworms developed normally and shed the inserted wire with the last larval or pupal skin. Also, no harmful effects of the gamma radiation have been observed in larvae which have had activity attached to them for a period of at least two weeks. Radioactive cobalt 60 has been chosen for this work because its high energy gamma radiation (1.10 and 1.30 Mev) makes it easy to detect under several inches of soil, and because its long half-life (5.3 years) minimizes corrections for decay and thus permits observations over a long period of time.

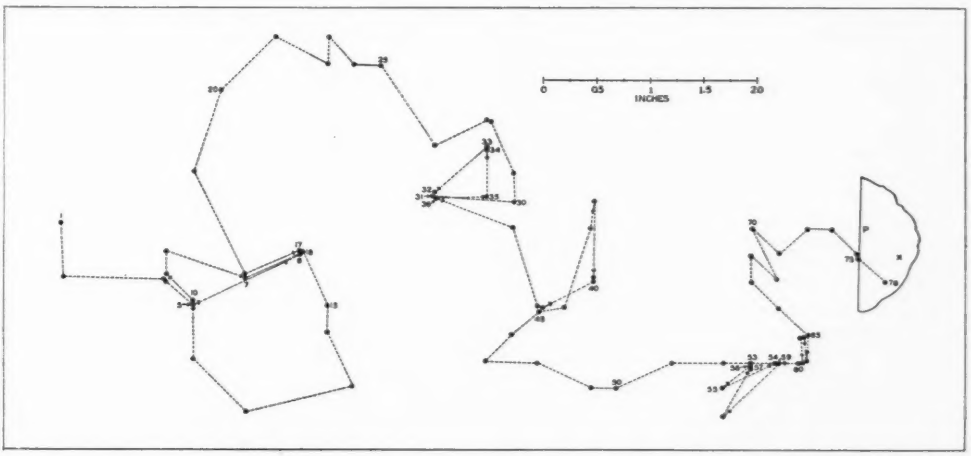

FIG. 1. Movement of a larva of *Ctenicera aeripennis destructor* (Brown) : diagram of movement in horizontal plane; the dots represent the positions of the larva at consecutive readings.

[1] Contribution No. 2617, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada, in co-operation with the Department of Chemistry, University of Saskatchewan. The assistance of the National Research Council of Canada in supplying radiocobalt for the work and in the use of certain equipment is gratefully acknowledged.

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[3] Senior Agricultural Assistant, Dominion Entomological Laboratory, Saskatoon.

[4] Head, Department of Chemistry, University of Saskatchewan.

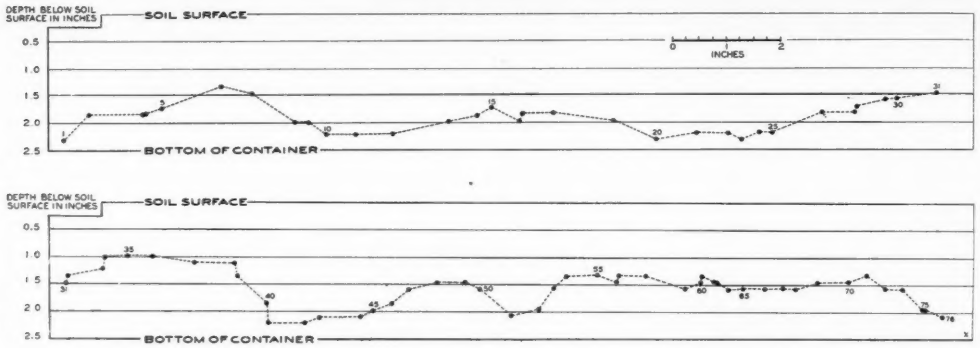

FIG. 2. Movement of a larva of *Ctenicera aeripennis destructor* (Brown) : graph showing relationship of vertical and horizontal distances traveled. (The scale is only one-half of that used in Fig. 1, but the horizontal distances between points 1 and 2, 2 and 3, et cetera, represent the same distances as separate these points in Fig. 1.)

Since radiocobalt wire was not yet available, preliminary tracing of wireworm movement was done by placing a small piece (approximately 2 mg) of radioactive cobalt metal in the caudal notch of the larva and attaching it to the urogomphi and ninth segment with vinylite plastic. Once activated or "tagged," the larva was followed by using a Geiger tube, designed to count gamma radiation, in the form of a movable probe connected to a counting rate meter giving an almost instantaneous measure of the intensity of radiation at any given point. By moving the Geiger probe over the soil surface and determining the position of maximum counting rate, the wireworm was localized in the horizontal plane. By previously calibrating the instrument for varying soil depths, using a standard activity, the absolute counting rate of this maximum was used to locate the wireworm in the vertical plane. This is the first recorded method of following both horizontal and vertical movement at the same time.

By this method the movement of a wireworm has been successfully charted. One of the observations is illustrated in Figs. 1 and 2, in which the movement of a large (13-mm) larva of *Ctenicera aeripennis destructor* is depicted. Fig. 1 reveals the direction of movement, in the horizontal plane; Fig. 2 disregards direction and simply relates the horizontal and vertical components of the path traveled. In conducting this investigation, the larva was placed in one end of an 8 in. × 6 in. container filled with soil to a depth of 2½ in., and with a potato piece (P) at the opposite end. The temperature was 22° C, and the soil moisture was approximately 20% (oven-dry basis). Readings were taken every 10 min, but the general progress of the larva was under observation at all times. Numbers 1 and 76 represent the first and last readings, respectively. Six and one-half hours after the last reading, the larva had moved to position X inside the potato piece.

It is planned to extend this preliminary work and to study the movement of the larva in response to such factors as temperature, light, soil moisture, soil type, soil firmness, and general condition, as well as various foods and chemicals. Movement of a larva in respect to other larvae (group behavior) might also be studied. In addition, the effect, if any, of the radioactivity on the wireworm will be observed. The technique is not restricted to wireworms and it is planned that the movements of other soil-inhabiting insects, particularly certain species of cutworms, may also be studied.

A knowledge of such responses should be of material assistance toward the understanding of fundamental wireworm biology and behavior, and would thus be of use in developing sampling techniques and in improving both cultural and chemical control methods.

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Adrenal Cortical Hormones: Analysis by Paper Partition Chromatography and Occurrence in the Urine of Normal Persons[1]

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The technique of paper partition chromatography (*2*), modified by the use of nonaqueous solvent systems, has

[1] This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

[2] National Cancer Institute Postdoctoral Fellow.

been found suited to the microanalysis of adrenal cortical hormones.

The relatively high solubilities of corticoids in polar solvents and their easily detectable α-ketol group rendered unnecessary their conversion to Girard's Reagent T

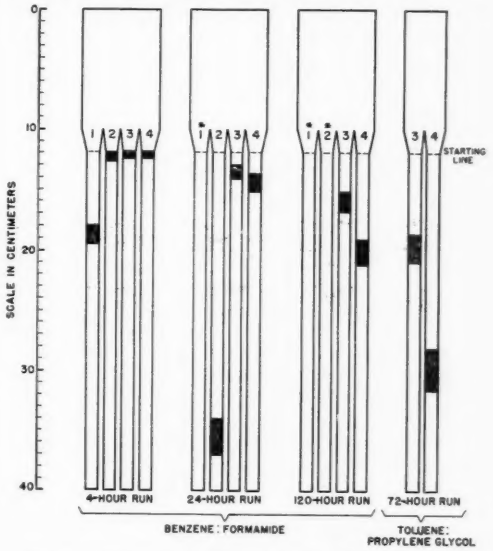

FIG. 1. Diagrams of chromatograms of pure corticoids in the two solvent systems. 1) Desoxycorticosterone; 2) Kendall's Compound A; 3) 17-hydroxycorticosterone; 4) Kendall's Compound E.

The asterisk indicates that the compound has run off the paper in the time shown.

hydrazones, used previously in steroid chromatography (*1, 4*). Of the many solvent combinations tried, the systems benzene:formamide and toluene:propylene glycol proved most satisfactory.

Impregnation of the strips with formamide or the glycol, prior to chromatography, was found necessary. The strips were dipped in the polar solvent and the excess removed by rolling them in an ordinary clothes wringer between dry filter paper. The samples were then applied to the strips and the chromatograms developed by the descending method with benzene or toluene saturated with its respective polar solvent. The papers were then dried in a stream of warm air and the positions of the steroids revealed by treatment with alkaline silver nitrate[3]. As little as 10 to 15 μg of a corticoid could be detected in the developed chromatogram. Kendall's Compound E, but no other corticoid tested, also gave an intense blue color in amounts over 15 μg when the chromatogram was sprayed with a 0.3% solution of iodine in 5% potassium iodide.

To allow precise comparison of unknown with known compounds, and also to limit sidewise diffusion of spots, samples were run on papers having two or more narrow limbs, as shown in Fig. 1.

Relatively long periods of development with these solvents were necessary to accomplish suitable separation

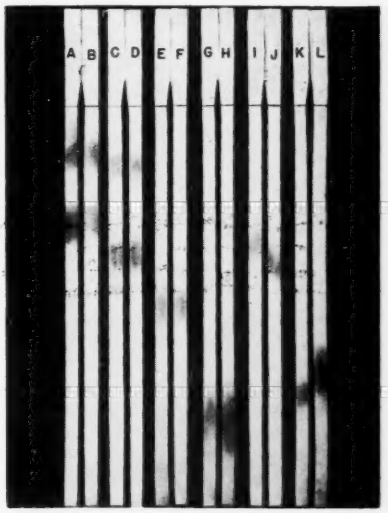

FIG. 2. Photographs of chromatograms of a urinary $C_{21}O_5$ fraction, pure compounds, and their derivatives. *A and B: 120-hr run in benzene:formamide.* A, 24-hr urine fraction; B, Kendall's Compound E (lower spot) and 17 hydroxycorticosterone (upper spot), 30 μg each. *C and D: 72-hr run in toluene:propylene glycol.* C, 24-hr urine fraction; D, Kendall's Compound E (lower spot) and 17 hydroxycorticosterone (upper spot), 30 μg each. *E–H: 15-hr run in benzene:formamide.* E, acetate of upper urinary compound in 24-hr aliquot; F, 17 hydroxycorticosterone acetate, 30 μg; G, acetate of lower urinary compound in 24-hr aliquot; H, Compound E acetate, 30 μg. *I–L: 8-hr run in benzene:formamide.* I, propionate of upper urinary compound in 24-hr aliquot; J, 17-hydroxycorticosterone propionate, 30 μg; K, propionate of lower urinary compound in 24-hr aliquot; L, Compound E propionate, 30 μg.

of the closely related cortical steroids. Since benzene and toluene moved rapidly and overflowed the papers, R_f values could not be computed. Hence the absolute movement of unknowns was compared with that of pure compounds run beside them on the same paper.

The behavior of the pure cortical hormones at our disposal[4] in the solvent systems used is illustrated in Figs. 1 and 2. Based on results with adrenal cortex extracts, Kendall's Compound A, and desoxycorticosterone, the benzene: formamide system seemed best adapted for the preliminary fractionation of complex corticoid mixtures and resolution of individual $C_{21}O_5$ compounds. This solvent system also proved useful in the analysis of the acetates and propionates of Kendall's Compound E and 17-hydroxycorticosterone. The toluene:propylene glycol system, on the other hand, gave wider and more

[3] Ten ml of 0.1 *N* $AgNO_3$, 10 drops of concentrated ammonium hydroxide, and 5 ml of 10% NaOH were mixed shortly before use. After maximum color production, the papers were rinsed in a 5% solution of $Na_2S_2O_3$ and then in water.

[4] Generously donated by Drs. E. C. Kendall, C. D. Kochakian, M. H. Kuizenga, H. L. Mason, E. Oppenheimer, and C. R. Scholz.

rapid separation of individual $C_{21}O_5$ compounds. The high degree of resolution obtained with these solvent systems is demonstrated in Fig. 2 by the wide separation of Kendall's E and 17-hydroxycorticosterone, both free and esterified, and also by the marked difference in movement of the acetates and propionates of each of these compounds.

Urine specimens, representing 72 or 96 hours' excretion, from 5 normal persons (4 males and 1 female), were continuously extracted at pH 1 with ether. The extracts were washed with dilute alkali and acid and then separately analyzed. By means of preliminary chromatograms in benzene:formamide, these neutral extracts were separated into three fractions containing, respectively, any C_2O_5, C_2O_4, or $C_{21}O_3$ corticoids that might have been excreted. Each $C_{21}O_5$ fraction was then rechromatographed beside pure compounds in toluene:propylene glycol. Two compounds whose movements were matched by Kendall's E and 17-hydroxycorticosterone, respectively, were found. Illustrations of these findings are given in Fig. 2 (A–D). These results were substantiated in all normals studied. The material eluted from appropriate areas of chromatograms not treated with the color reagent presented the ultraviolet absorption curve (maximum absorption at 240 mμ) typical of α,β-unsaturated 3-ketosteroids. The urinary compound whose mobility was the same as that of Kendall's Compound E also gave the blue color wit the iodine reagent. The other urinary compound, when eluted from the paper and dried, gave the yellow-green fluorescence with concentrated H_2SO_4 described for 17-hydroxycorticosterone (*3*). The eluted urinary compounds were also subjected to procedures for the preparation of the acetates and propionates of Kendall's Compound E and 17-hydroxycorticosterone and were then chromatographed beside the corresponding authentic esters. The alkaline silver solution was used as the color reagent. The derivatives of the urinary compounds moved at the same rates as the esters of the pure steroids (Fig. 2, E–L).

By rough matching of the size and intensity of the urinary spots with those of known quantities of pure Kendall's E and 17-hydroxycorticosterone, the normal 24-hr excretion of each was estimated to be between 20 and 40 μg. This was substantiated by measurement of the optical density at 240 mμ of the eluted compounds. In the normal persons studied, the excretion of Kendall's E appeared to be slightly greater than of 17-hydroxycorticosterone.

In the $C_{21}O_5$ fractions only the two compounds were found in 24-hr aliquots of the urine extracts. Thus any other steroid of this group, if present, was excreted in daily amounts under 10 μg, the minimum amount detectable with the alkaline silver reagent. No $C_{21}O_4$ or $C_{21}O_3$ corticoids could be found in aliquots of these extracts representing up to 72 hours' excretion.

In conclusion, the presence of Kendall's Compound E and 17-hydroxycorticosterone in the urine of normal persons is strongly indicated by: 1) the agreement in chromatographic behavior of the urinary compounds and the known steroids; 2) the similar movements of their esters; 3) the characteristic color reactions, and 4) the typical ultraviolet absorption curves. No other cortical hormone was found in appreciable amounts. The solvent systems described may be of value in the purification of synthetic cortical hormones by partition chromatography in starch or filter paper pulp columns.

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Cultivation of *Endamoeba histolytica* with *Trypanosoma cruzi*

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Endamoeba histolytica has been cultivated *in vitro* since 1925 (*1*), but always in the presence of bacteria. Cultivation of the ameba in the absence of bacteria is of utmost importance, since bacteria-free cultures are prerequisite for basic studies on the physiology and pathogenesis of this parasite, and on the serodiagnosis of amebiasis. During recent years several notable improvements in cultivation techniques have been accomplished, resulting in the acquisition of new information on growth requirements of the ameba. By the use of a microisolation apparatus, Rees and his associates were able to free cysts of *E. histolytica* from contaminants and thus to establish cultures of the ameba with various single species of bacteria (*2, 5*). Cultures with one species of bacterium designated as organism *t* have been used for preparation of antigens of high potency and specificity for the complement fixation test for amebiasis (*6, 12*). In addition, some information concerning the vitamin-cholesterol requirements of *E. histolytica*-organism *t* was obtained with the use of diphasic egg white medium and dialyzate medium (*6, 7, 13*). The work of Jacobs (*3*) demonstrated the usefulness of antibiotics in eliminating bacteria from ameba cultures and thus provided a basis for testing the effects of growth factors. Heat-treated *Escherichia coli* supported some growth of the ameba after the associated bacterium *Clostridium perfringens* had been eliminated with the use of penicillin and serial transfer. More recently, the work of Shaffer and Frye (*11*) demonstrated that good growth of the ameba may be obtained in wholly liquid medium with a species of streptobacillus inhibited with penicillin. The streptobacillus with an inhibiting dosage of penicillin was added at each serial transfer. The cultures of *E. histolytica* with live and heat-treated *Trypanosoma cruzi*, as described in the present report, were obtained by substituting the trypanosome for the bacterial associates used by previous investigators.

The growth of *E. histolytica* with *T. cruzi* was demonstrated in test tubes and in microtubes approximately

4 × 50 mm (*9, 10*). In both types of tubes a petrolatum seal was used to prevent access of atmospheric oxygen to the medium. The medium was composed of a thioglycollate preparation (Baltimore Biological Laboratory), horse serum, and the overlay of NIH diphasic blood-agar cultures (*4*) containing a rich suspension of *T. cruzi*. The F-22 strain of *E. histolytica* received through the courtesy of Drs. J. G. Shaffer and W. W. Frye, then of Vanderbilt University, was used in all experiments.

Test tubes containing the medium described were inoculated with amebas from cultures grown in the presence of penicillin-inhibited streptobacillus in a liquid thioglycollate-serum medium. Penicillin was used in the ameba-trypanosome cultures during the first four serial transfers to insure elimination of the streptobacillus and sterility tests were conducted at each transfer; these were negative. Control tubes without *T. cruzi* showed no growth of amebas. The ameba-trypanosome cultures have been maintained through 15 transplants at 48-hr intervals without any diminution in growth of the amebas. Rich cultures of amebas were produced in 48 hr and transplanted at the rate of six tubes from one. Active trypanosomes have been found to persist for as long as 10 days in these tubes, but clumping of the flagellates indicates that this medium is not entirely satisfactory for their growth.

The microcultures of *E. histolytica* were initiated from single trophozoites, washed free from components of the medium in sterile Locke's solution, and transferred by microisolation to microtubes containing the same medium as used in the test tubes, with the trypanosomes. As many as 200 amebas, the progeny of a single trophozoite, were counted by direct microscopic examination of the microtubes at 72 hr of incubation. Active amebas were seen for 10 days. Serial transfer of the microcultures was not carried out but the medium was transferred after 10 days to thioglycollate and neopeptone-broth media for tests of bacterial sterility. No growth of bacteria occurred in these tests.

Growth of *E. histolytica* with heat-treated *T. cruzi* has been demonstrated only in microcultures. The trypanosomes in the overlay of NIH blood-agar cultures were exposed to temperatures of 46° C for 1 hr 15 min in a water bath before addition of other constituents of the medium. Each microtube was inoculated with a single ameba trophozoite. In 20 microcultures thus far established, the ameba reproduced as rapidly as with non-heated *T. cruzi*. Transfer of the entire contents of the microtubes to NIH blood-agar medium gave no growth of trypanosomes.

The principal advantage of *E. histolytica-T. cruzi* cultures over ameba-bacteria cultures lies in the fact that *T. cruzi* may be inactivated at temperatures only a little higher than 37° C and hence without as much deleterious effect on proteins and enzyme systems as occurs in the heat-killing of bacteria. The cultures may therefore serve as a basis for the identification of growth factors required by *E. histolytica*.

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A Micromethod for Fat Analysis Based on Formation of Monolayer Films

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Lord Rayleigh (*5*) found in 1890 that lipids spread on water form films of wide extent, and Langmuir (*4*) found the water-insoluble fatty acid able to spread in monomolecular layers, with each molecule occupying an area of 20.5 square angstrom units, independent of the number of carbon atoms in the molecule.

Adam (*1*) showed that the spread of the film is little affected by temperatures from 20° to 30° C, if a film tension of 20 dynes per cm² is maintained.

The sterols, glycerides, phosphatids, cerebrosides, and hydrocarbons likewise spread, and the molecules occupy from 20 to 120 sq A. Mixed fats spread to about the same extent. Under controlled conditions, the area of this spread is a sensitive measure of the amount of fat. Gorter and Grendel (*3*) used this criterion to estimate the lipids of red cells.

It is relatively simple to estimate fatty acids, with a sensitivity and accuracy beyond other methods, by this spreading technique. A circular glass dish, 7½ in. in diam and ¾ in. deep, is supported on a small leveling table placed in an enamel tray, 12 × 14 × 1¾ in. The glass dish is coated with hard paraffin and filled with 0.3% H_2SO_4 (pH 1-2) until the level is above the edge of the dish.

The surface of this solution is cleaned by passing a glass slide (10 × 1 × $\frac{1}{16}$ in.) from one edge to the other of the glass dish several times, wiping it dry each time. Droplets of piston oil are placed on the clean surface. They spread over the surface with a play of colors. Oil is added until the color is green. Such a film has been shown to have a uniform tension of 20 ± 2 dynes/cm². The piston oil is a good grade of lubricating oil heated to 300° C for 8 hr until it spreads as a monolayer (*2*).

The fat to be estimated is extracted from the original source by any good procedure that presents all the lipids in a clear petroleum ether solution in a volumetric flask of from 0.5 to 25 ml. From this volumetric flask a definite aliquot is placed by means of a capillary pipette

in the center of the piston oil film. As the solvent evaporates, the liberated fat spreads as a film against the pressure of the heavy oil film until equilibrium is reached.

A pane of glass is placed on the enameled tray, clearing the water surface by approximately ¼ in. A timer is started as soon as the fat solution is added. The film is allowed to age for 3 min. At the end of this time, by means of a wax pencil, the boundary of the film between the fat and piston oil is traced on the glass surface. A light placed directly above the dish aids in making this boundary distinct. The outline on the glass plate is readily traced on coordinate paper and the area is obtained by counting the squares; or if plain paper is used the outline is traced with a planimeter.

TABLE 1

Amount taken (in g)	Amount spread (in ml)	Spread (in sq in.)	Fat (equiv. in g)	Total fat found (in g)
0.0008	0.0052	3.6	0.0000054	0.00085
0.0018	0.0052	7.24	0.00001096	0.00173
0.0073	0.0052	31.28	0.00004692	0.00739

In the determination of small amounts of fat care must be taken in measuring the petroleum ether solutions. Small pipettes (containing from 0.005 to 0.1 ml) are used, that have narrow constrictions at the tip and at the calibrating mark to slow the flow of the ether. These are calibrated with mercury.

TABLE 2

Molecular weight	Area molecule (sq A)	Theoretical (1 sq in. = μg)	Found
Palmitic acid 256	20.5	1.33	1.35
Stearic acid 284	20.5	1.50	1.46
Cholesterol 390	41.0	1.02	1.05

In use, the pipettes are filled above the mark. The tip is wiped on filter paper and then held against the paper until the solution is brought to the mark. The solution is delivered on the surface with the last droplet forced out under the surface. The pipettes are rinsed with ethyl ether and dried with warm dry air in order to remove traces of moisture that render the measurement inaccurate. Repeated spreading of the same amount of lipid checks within 2%. This covers the pipetting of sample, the tracing, and the planimeter readings. Twenty-one such spreadings at 28° C gave a variation in areas of 1.6 ± .14% with a standard deviation of 0.96.

The sensitivity of the method is shown by Table 1, showing the analysis of skin fat, which was dissolved in 0.82 ml of petroleum ether. Aliquots were drawn with a pipette delivering 0.0052 ml. The factor was 0.0000015 g/sq in.

The important constituents of skin fat are fatty acids and sterols. These should agree with the theoretical spread. Using 20.5 sq A per molecule for fatty acids, and 41 sq A for cholesterol, the agreement is as shown in Table 2.

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A Preliminary Report on the Role of the Pineal Organ in the Control of Pigment Cells and Light Reactions in Recent Teleost Fishes[1]

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Although there has been a considerable amount of speculation on the possible functioning of both the pineal and the parietal organ in various fossil vertebrates, very little experimental work has been done on recent forms. Moreover, the precise function of the pineal organ in recent teleosts has never been clearly elucidated, although the structure is evidently present in all. A recent survey of the knowledge of the organ has been given by Gladstone and Wakeley (*5*). In fishes, all that is known concerning the relation of the pineal organ to pigment control is based on the work of von Frisch (*4*), which was confirmed by Scharrer (*7*). They demonstrated that the granules in melanophores of *Phoxinus* would disperse if the pineal region was stimulated by light. Since von Frisch was able to demonstrate that light sensitivity was not completely removed by extirpation of the pineal, and since he considered the lining of the ventricle of the diencephalon to have sensory cells, the exposed portions of the brain that react to light are here referred to as the pineal area. Parker (*6*) considered this fish exceptional. Scharrer demonstrated also that the pineal had an influence on phototaxis as well as on pigmentation, as did Young (*8*) for the very different, nonteleost, *Lampetra*. Breder and Rasquin (*3*) demonstrated an influence of the pineal on phototaxis for the blind cave fish *Anoptichthys*, which is responsible for the sign of the reaction.

Since the last work was published it has been possible to demonstrate that in a variety of eyed fishes dwelling in light, the pineal area has a distinct influence on both the behavior of the melanophores and the reactions of the fishes to light and darkness. It has been found that the fishes studied divide into three groups: those species in which the tissues, including the skull, overlying the pineal are sufficiently transparent or translucent to permit light to enter the cranial cavity freely over this region, as illustrated in Fig. 1; those in which the tissues

[1] This study will be reported in full detail at a later time. Most of the work was carried on at the Lerner Marine Laboratory at Bimini, and was in part supported by a grant from the American Cancer Society.

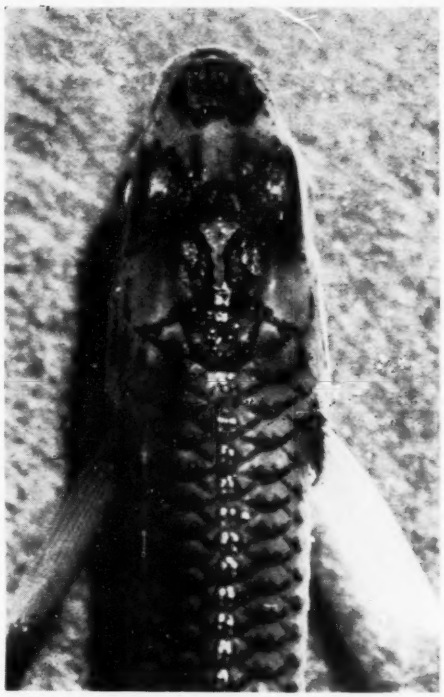

FIG. 1. Photograph of the top of the head of *Atherina* (*Hepsetia*) *stipes* Muller and Troschel, showing the clear median area immediately behind the eyes unprotected by chromatophores directly over the pineal body. Magnification × 3.4.

are apparently too opaque to permit such entry of light; and finally, those in which the behavior of appropriately placed chromatophores control this condition.

To a very large extent, these three morphological conditions are associated with the manner in which the fishes react to light. Species which have the most extremely exposed pineal areas are strongly light-positive, whereas those that are the most protected are among the most light-negative. The fishes whose chromatophores regulate the extent of exposure of the pineal area by the dispersion or concentration of pigment granules present what appears to be an erratic behavior in regard to their reactions to light. This is apparently owing to the functioning of these structures covering the brain, the details of which are as yet to be worked out. Certain fishes with rather complicated structures dorsal to the pineal area do not fit readily into this scheme, but they are under study and it is becoming apparent that each species has its own set of peculiarities. These, however, in no way alter the fundamental fact of pineal control in numerous species in which the effects are clearly apparent.

Light reactions were demonstrated by presenting the fishes with the choice of a black-surfaced and covered compartment or a white-surfaced and lighted compartment, as described by Breder and Rasquin (*3*). The results of these choices were compared with the morphology of the pineal covering. Species showing the three types of behavior are listed here:

Species with exposed pineal areas that are light-positive. The figures give the extent of positivity in terms of percent of time spent in the light or darkness, with zero equal to light indifference.

Jenkinsia lamprotaenia (Gosse)	+100.0
Sardinella macropthalmus (Ranzani)	+ 99.8
Leptocephalus larvae Sp.?	+ 26.0
Brachydanio rerio (Ham.-Buchanan)	+100.0
Atherina[2] *stipes* Muller & Troschel	+100.0
Carapus bermudensis (Jones)	+100.0

Species in which chromatophores control the coverage of the pineal areas and show changeable reactions with regard to light. These cannot be given numerical treatment because the methods employed demand a certain amount of swimming by the fishes.

Sphyraena barracuda (Walbaum)
Strongylura notata (Poey)
Hemirhamphus brasiliensis (Linnaeus)
Synodus synodus (Linnaeus)

Species with permanently covered pineal areas that are light-negative. The figures give the extent of negativity in terms of percent.

Astyanax mexicanus (Filippi)	−58.7
Ameiurus nebulosus (LeSueur)	−99.3
Haemulon melanurum (Linnaeus)	−93.5
Thalassoma bifasciatum (Bloch)	−33.0

Species that for one reason or another do not subscribe to the preceding three simple conditions.

Light-positive but with well-covered pineal area

Eucinostomus gula (Cuvier & Valenciennes)	+ 43.5
Pseudupeneus maculatus (Bloch)	+100.0
Apogonichthys stellatus Cope	+ 30.5

Practically light indifferent and pineal area covered

Cyprinodon baconi Breder	+ 9.3

Light negative with exposed pineal area

Mugil trichodon Poey	− 53.5

Covered pineal area but behavior indeterminate
Bathygobius soporator (Cuvier & Valenciennes)

The irregularities shown in the last series should not be surprising when it is recalled that teleost fishes existent today represent long independent evolutionary lines and in many of their numerous aspects show striking variations in practically all their morphological and behavioral features. That better than two out of three of all fishes examined fit into this scheme is probably the most remarkable feature of this study. It is to be noted too that there is a strong semblance of phylogeny inherent in these lists. The exposed pineal group is represented by all nonacanthopterygian fishes except for *Carapus*, which is a highly specialized member of the Ophidioidea, and *Atherina*, one of the Percesoces. The rest are either Isospondyles, Apodes, or Ostariophysi. The group with a controllable cover are also largely nonacanthopterygian, being represented by members of the

[2] *Membras* according to the revision of L. P. Schultz, *Proc. U. S. Nat. Mus.*, 1948, **98**, pp. 39–40.

Synentognathi except for *Synodus*, an Isospondyle, and *Sphyraena*, one of the Percesoces. Two of the group with covered pineals are Acanthopterygii, and two, *Astyanax* and *Ameiurus*, represent the Ostariophysi. The inconsistent group is all acanthopterygian except for *Cyprinodon*, one of the Cyprinodontes. This represents a clear reduction of pineal influence as one reaches the spiny-rayed fishes, and their relative independence from that influence may be seen from the following tabulation:

Group	1	2	3	4
% Acanthopterygii	33	25	50	83
Mean of light reactions %	+87.6	..	−71.1	+47.4

That there is a somewhat parallel difference in this matter in ontogeny as well, is to be expected, as has previously been suggested by Breder and Rasquin (*3*). It also follows that without special and very elaborate morphological provision, larger fishes have better coverage of the pineal than smaller ones because of the greater thickness, in an absolute sense, of the tissues over the brain. Also a sexual difference in the extent of the negative light reactions of the very sexually dichromatic *Thalassoma* became evident, the females being markedly less light-negative than the more heavily pigmented males. The nocturnal *Apogonichthys* was found to be less light-positive when tested at night than in the daytime. These items indicate some of the numerous factors which must be explored more fully. In cases where duplicate tests were made, only the figures less favorable to the thesis herewith developed have been used.

In addition to the stimuli received by way of the eyes and the pineal area, there is evidently a direct effect on some other receptors, possibly on nerve endings in the skin or deeper in the translucent flesh of some of these fishes. Even with blinding and covering the pineal, it was found that phototaxis in *Atherina* was still light-positive, but to a lesser degree, whereas *Eucinostomus* became light-negative, and *Haemulon* became less light-negative. The numerical values for this experiment follow, the values being expressed in percent of light-positiveness:

Species	Normal	Blind	Blind and pineal covered
A. stipes	+100.0	+40.6	+12.5
E. gula	+ 43.5	+31.9	−17.5
H. melanurum	− 93.5	− 6.9	−24.0

Statistical analyses of the numerical values are to be fully reported later, but it may be pointed out that they are of high significance, the majority showing no overlap whatever.

The direct influence of the pineal area on pigmentation could be shown by covering with India ink the heads of fishes having exposed pineals. Fishes in the first category so tested all expanded their melanophores, whereas the effect was less extreme in the second category, the fishes showing dark patterns, in some cases not resembling any of the normal ones. Even fishes in the third category showed some effect and seemed to be prevented from entering their lightest phase. This evidently indicated that in the sizes available for experimental purposes there was still some radiant energy impinging on the pineal area through what seemed to be opaque covering.

Because fishes have been shown to react to an injection of adrenalin by concentrating their melanin granules, various species under study were so treated. All showed a reaction in that the meningeal melanophores and the iris melanophores concentrated their granules, but the results here contrasted with work previously recorded in that the dermal melanophores behaved differently in many species. Evidently the melanophores in these species are under strong nervous control which adrenalin, although administered in massive doses, could not override. Some fishes, in fact, showed very dark markings and only three, *Bathygobius, Opsanus,* and *Ameiurus,* showed the typical blanching recorded in the literature. Fishes such as *Haemulon melanurum* and *Chaetodipterus faber* (Broussonet), which react to a white background by becoming dark instead of blanching in the more usual manner, were especially marked in this regard. Further details concerning this opposed pigmentary behavior are given by Breder (*1, 2*).

Since the covering of the pineal area contains chromatosomes which consist of melanophores and iridophores, the simple concentration or dispersion of the granules of the melanophores is only one element in the shading of the pineal region. The deflection of light from the guanin crystals carried in the iridophores may have as much to do with shading the pineal area as does the dispersion of black granules in the melanophores. The meninges that cover the brain, exclusive of the pineal area, can be seen to be heavily invested with iridophores as well as melanophores. This is strikingly demonstrated by adrenalin injection in the more or less translucent fishes of the first group. The brain appears white a few seconds after injection, when the pigment granules of the melanophores are concentrated, and the crystals in the iridophores or leucophores either disperse or remain unaffected by the adrenalin. The contents of erythrophores, xanthophores, and iridophores were not concentrated by adrenalin, nor were they seen to be influenced by the pineal area, although they are obviously under nervous control.

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Cobalt Polycythemia and Cytochrome C[1]

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It has been reported by Proger and Dekaneas (*4, 5*) that intravenous administration of cytochrome C into both rats and men will reduce the effect of anoxemic conditions, e.g., reverse the effect of a 10% oxygen atmosphere and change the electrocardiographic pattern to normal after myocardial anoxemia. Zelman and Gilbert (*7*) noted a case of hepatolenticular degeneration in man, presenting indications of tissue anoxemia, which responded to the administration of cytochrome C. Scheinberg and Mitchel (*6*) found that administration of cytochrome C did not prolong the life of rats in an atmosphere of 2.8 or 3.9% oxygen. This concentration of oxygen is considerably below that used by Proger and may be a limiting concentration. On the other hand, it has been reported recently by Beinert and Reissmann (*1*) that cytochrome C labeled with radioactive iron did not enter the tissue cells of rats following intravenous injection.

TABLE 1

EFFECT OF CYTOCHROME C ON COBALT-INDUCED POLYCYTHEMIA OF THE RAT

Supplement	Number of animals	Hemoglobin—g/100 ml		
		Before supplement	Weeks after supplement	
			1	2
None	9	14.2 ± 1.0*	14.9 ± 0.9	13.4 ± 0.8
Cytochrome C† .	6	13.5 ± 0.5	13.8 ± 0.9	13.4 ± 0.6
Cobalt‡	7	18.3 ± 0.9	18.2 ± 0.9	17.6 ± 0.9
Cobalt‡ plus cytochrome C†	8	18.8 ± 0.8	18.2 ± 0.9	18.4 ± 0.9

* Mean plus standard deviation.

† Animals receiving cytochrome C were given 7.5 mg per day intravenously for the test period.

‡ Cobalt was given intraperitoneally, 0.5 mg per day.

It has been suggested (*2*) that the polycythemia produced by cobalt may be a compensation to internal anoxemia produced by the inhibition of cellular respiration systems. It was considered of interest to determine whether intravenous administration of cytochrome C would counteract this effect.

Polycythemia was produced in hooded rats maintained on dog chow by the intraperitoneal injection of 0.5 mg of cobalt per day 6 days a week. When the level of hemoglobin became constant, the test animals received 7.5 mg of cytochrome C, dissolved in physiological saline, into one of the tail veins. This amount of cytochrome C is approximately one half that contained in the body of the rat, according to Drabkin and Crandall (*3*). Cobalt was given to the test animals during the entire study.

Results are presented in Table 1. The intravenous administration of cytochrome C did not appear to affect the hemoglobin concentration in either normal animals, those not receiving cobalt, or polycythemic animals. Perhaps this is to be expected in light of the work of Beinert and Reissmann in which intravenously administered cytochrome C did not appear to enter the tissue cells.

[1] The cytochrome C used in this study was generously supplied by Dr. C. E. Graham of the Wilson Laboratories, Chicago, Illinois.

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Rapid Method of Preparing Schiff's Reagent for the Feulgen Test

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The following reagents are commonly used to reduce fuchsin base to the leuco compound: sodium bisulfite, potassium metabisulfite, sodium thiosulfate, and sulfur dioxide (*1, 2*). To obtain satisfactory results with these reagents, the solutions should stand for about 24 hr. We have found that by the use of a readily obtainable reagent, sodium hydrosulfite, Schiff's reagent may be prepared in a few minutes. A summary of the procedure follows:

Prepare a 0.5% solution of fuchsin base as usual. To 100 ml of this solution add 0.5 g of active and undecomposed sodium hydrosulfite, and at the same time add 0.25 g of activated charcoal (Nuchar). Shake thoroughly in a closed flask, and filter through a coarse filter paper. The finished product is of a light amber tinge.

The tissue section to be examined is hydrolyzed in normal HCl for 4 min at 60° C, washed, and allowed to stand in the leuco-base solution for 2 hr. To prevent oxidation, the solution is preferably layered with xylol. The slides are then washed, first in a 0.25% solution of sodium hydrosulfite, next with distilled water; then they are cleared and mounted.

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Comments and Communications

Discordant Time Scales

The superposition of one time scale on another may produce weird results. Dating the birth of the moon and the attendant and consequent terrestrial pangs in the Mesozoic era will make most geologists sit up and take notice. On the other hand, the dating as Miocene, or earlier, of the upheaval of mountains which have since lost little or none of their altitude under the attack of erosional processes sometimes passes unnoticed, though it also must be regarded as of doubtful authenticity if one is willing to adopt the modern scale, which pushes Miocene events back twenty to thirty million years.

For examples of such confusing time scales it will suffice to refer to two publications recently issued in Vienna by no less authorities than F. X. Schaffer, author of a classic three-volume textbook of geology, and the distinguished geographer, Johann Sölch.

Schaffer (*Der Mond und das Leben*, Vienna: F. Deuticke, 1948) advocates the Kant-Böhm-Schaffer theory that very considerable changes in the shape of the earth have taken place, even in late geological times, as a result of the slowing down of the earth's rotation due to tidal friction. He claims that these changes have been great enough to cause the catastrophic and more or less rhythmic tectogenies that have deformed the crust and also the periodic interchange of land and sea, as well as changes of altitude and of continental configuration capable of profoundly affecting geological climates.

Against such a theory are usually set the fact that earth tides are now minute and the dictum of astronomers that the rate of rotation of the earth is not now changing appreciably. Accepting the suggestion that the present rate of change in the length of the day is of the order of 1/200 sec per century (Poynting, J. H. *The earth*. Cambridge: Univ. Press, 1913) and without pretending to have a reliable basis for calculation, one might think of the resulting change in the oblateness of the earth as reducing the excess of the equatorial over the polar diameter by a few hundred feet at the most, in 50 to 100 million years. However wild this estimate may be, it is obvious that the change is not likely to be sufficiently great to produce spectacular results, and it is not surprising, therefore, that Schaffer has stood alone in his support (*Lehrbuch der Geologie, I*. Vienna: F. Deuticke, 1922; *Pan-American Geologist*, 1928, **50**, 121) of Böhm-Böhmersheim (*Abplattung und Gebirgsbildung*. Vienna: F. Deuticke, 1910).

The days of active tidal evolution are certainly much more remote than any one thought possible when, 70 years ago, G. Darwin formulated the theory of the lengthening of the day, with all its necessary consequences of change in the shape of the earth globe. Instead of between 50 and 60 million years, which was Darwin's estimate of the time that has elapsed since, as he supposed, the moon parted company with the earth, R. W. van Bemmelen (*Geologie en Mijnbouw*, 1949, **11**, 1–21) now allows nearly two thousand million years; and to this evaluation may be added at least a thousand million years more if A. Holmes' estimate (*Nature*, Lond., 1947, **159**, 127) of the age of the earth is acceptable.

Notwithstanding the obvious necessity of moving with the times to the extent of expanding Darwin's time scale in a consistent manner, Schaffer retains it without modification. In his latest work (*Der Mond und das Leben*, Vienna: F. Deuticke, 1948) this scale is superposed on an absolute timing of geological chronology which, though not quite in accordance with the most recent age determinations, is at least very much slower than any that was popularly approved when Darwin made his calculations, and the astonishing conclusion emerges that somewhere between late Carboniferous and Jurassic times (the 56 million years ago of Darwin) the earth rotated once every 6¾ hr. The author then resorts to extrapolation and by this means estimates that some time in the Algonkian (when a certain ameba took its 34-min pulsation period from the tidal interval on the birthday of the species) the earth was rotating once every hour. If it were possible to accept such evidence of spectacular slowing down of the earth's rotation in geological times, however caused, the Kant-Böhm-Schaffer theory would receive much support.

The story of the eastern Alps as unfolded by Sölch begins with moderate uplift of a very well-developed peneplain, which suffered some dissection and was then further warped up to its present summit altitude. So the tectonic form of the mountains has come to consist of northern and southern upheaved arches, between which is the Norie furrow, a broad strip of relatively depressed country carrying consequent east-west drainage. Though the warping is assigned the date of the assumed contemporaneous depression of the Vienna basin, which was flooded by a middle Miocene sea, dissection, while further advanced on weaker parts of the terrain, has failed as yet to break the continuity of the peneplain over much of the higher northern arch, for this is formed of a thick mass of resistant limestone.

Thus all the landscape evolution described might be pictured as spread over two or three million years, scarcely longer. Miocene events are now relegated to a past ten times more distant than this, however, and this perhaps warrants a suggestion that the peneplain here involved intersects and is of much later development than the land surface buried under the sediments of the Vienna basin. If it is not so, and if some 25 million years has been occupied by this mild warping upheaval and the development in a new cycle of mature erosional relief over only the weaker parts of the area affected, how much—how very much—longer a period will have to be allowed for the development of the earlier peneplain, which truncates varied structures as well as an enormous potential

relief, and for the Alpine folding in which that earlier complex structure and relief had their origin?

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A Suggested Method for Estimating Pre-Hunting Season Status of Scaled Quail Populations

It is desirable in game management to have practical, quantitative "yardsticks" which can be applied to game populations in order to aid the administrator in formulating well-advised harvesting regulations.

The following suggestion relative to estimating the population status of scaled quail (*Callipepla squamata pallida*) may have value, though admittedly there are no known instances in which it has been tried on an extensive scale. The type of range favored and the coveying habits of the species render it relatively easy for experienced field personnel to net scaled quail alive in numbers after the fall covey-gather. The grown young-of-the-year can easily be distinguished with great certainty from all other age classes by minor, but apparently constant, differences in the plumage. Hence, the age ratio thus established indicates the degree of success of brood survival in that year up to the time the observations were made. Brood survival, indicated by the ratio between young-of-the-year and adults, presumably is a reliable index of the state of vigor of the population as a whole, and should therefore also be a good index of the actual abundance of the species when large areas are considered, except in grossly underpopulated range. It is now well established that in quail, as in other upland game birds, it is the bird-of-the-year that provides most of the shooting (Steen, M. O. *Trans. 9th N. A. Wildlife Conf.*, 1944, 331).

For the six to eight weeks prior to date of setting the quail hunting regulations—say, from about the middle of September to the middle of November—a great deal of pertinent information could be secured by one or more two-man crews, each provided with two jeeps equipped with small hauling trailers, if these crews are assigned to net and examine as many quail as possible in as many key areas as possible of the administrative unit concerned. It should then be possible to ascertain the actual age ratio among a sizable sample of the quail population. The netted quail, of course, could be released unharmed at the trapping site as soon as they had been processed.

The technique of netting quail by means of the modified fyke net with long, V-shaped wings of cord netting is probably too well known among game men to merit detailing here. Suffice it to say that whole coveys may often be maneuvered into such nets by two or three experienced men, on foot, on horseback, or with the aid of vehicles. Trapping the birds in fairly large numbers is unquestionably, a practical matter. J. S. Ligon (in a paper published by the New Mexico Game and Fish Commission, Santa Fe, 1946) gives particularly complete directions as to this procedure.

A. C. Bent (*Bull. U. S. nat. Mus.*, 1932, 162, 54) states that scaled quail undergo a complete molt in August and September, at which time the young-of-the-year become practically indistinguishable from the full adults except for the retention of the first two juvenile primaries in each wing. The writer has found this criterion difficult to apply, but fortunately there is another easier and possibly more accurate method (Leopold, A. S. *J. wildlife Management*, 1939, 3[3], 261; and FIGGE, H. *Proc. 26th ann. conf. west. ass. state game fish commissioners*, 1946, 161). This method depends on the retention of the juvenile primary covert feathers until the bird is well into its second summer (age 15–18 months). These juvenile primary coverts are conspicuously mottled with a whitish or buffy color. In the fully mature adult condition, assumed after the first postnuptial molt, they are plain bluish gray. This method of distinguishing age classes is considered to be extremely accurate, and has been used personally by the writer in quail investigations in New Mexico with very satisfactory results. Incidentally, it appears to work as well for Gambel's quail (*Lophortyx gambeli*) and Texas bob-whites (*Colinus virginianus texanus*) as for scaled quail. The method is used as standard procedure for aging quail at the New Mexico State Game Farm (personal communication from Superintendent James L. Cox). Furthermore, Ligon, an outstanding authority on upland game, has also used this method extensively in aging quail and considers it to be extremely reliable (personal communication).

The age ratio is easily secured from the raw data. For example, a ratio of 1000 young to 1000 adults would give an age ratio of one young to each adult, or two young to each pair, assuming a 1:1 sex ratio and all birds breeding. Such an age ratio over a large area in the fall before the hunting season would indicate poor brood survival, and presumably a general unthrifty condition of the quail population. A restricted season and bag limit would apparently be indicated for that year. On the other hand, suppose the raw data to be 1500 young to 500 adults. The resulting age ratio would be three young to each adult, or six surviving young to each pair. This would indicate rather good brood survival, and presumably a thrifty condition of the quail population. A longer season and a more liberal bag limit could accordingly be endured by the quail without unduly cutting into next year's breeding stock.

Besides securing age class data, this suggested procedure could also be made to yield extremely valuable basic data as to prevailing sex ratios and, by making banding of each bird trapped a routine part of the procedure, could aid in gradually building up very useful statistics relative to survival and movements of the banded birds.

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News and Notes

Report on Como Conference on Cosmic Radiation

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From September 11 to 16 some of the world's foremost physicists gathered at Como (Italy) to celebrate the 150th anniversary of Volta's discovery of the electric pile. The main topic of discussion was the field of cosmic radiation, with particular emphasis on its elementary particle aspects. The conference was organized by the Italian Physical Society, under the sponsorship of the International Union of Physics (Unesco) whose president, H. A. Kramers (Holland), originally suggested the idea of holding an International Colloquium on Cosmic Rays this year. G. Polvani (Milan), president of the Italian Physical Society, presided over the conference and he, together with E. Amaldi and G. Bernardini (Rome) were the chief organizers of the scientific meetings.

During the conference a number of social events took place which were characterized by extraordinary hospitality and cordiality extended to all participants by the Italian scientists, the Italian government, and Italian industry. Every one of us who attended the conference will vividly remember the great informality of the meetings and the cordial atmosphere among physicists traveling to Como from all over the world.

The Volta Celebration at Como was preceded by a Conference on Nuclear Physics and Electrodynamics held in Basel from September 1 to 10. These two conferences represented the first really large international gatherings of physicists since World War II. The meetings demonstrated that an exchange of ideas is badly needed, and that true cooperation between scientists is of the greatest importance for the future progress of science.

More than 400 physicists attended the Como conference. A few of their names might make for orientation: E. Fermi, I. I. Rabi, J. Schwinger, E. M. McMillan, R. B. Brode, E. Segre, G. Placzek, and M. Schein (United States); G. Polvani, E. Amaldi, G. Bernardini, and B. Ferretti (Italy); W. Pauli, P. Scherrer, P. Huber, and F. Fierz (Switzerland); J. Clay and H. A. Kramers (Holland); G. P. Thomson, P. M. S. Blackett, C. F. Powell, J. G. Wilson, G. D. Rochester, and D. E. Perkins (England); L. Leprince-Ringuet, M. Morand, J. Daudin, R. Maze, and A. Rogozinski (France); W. Heisenberg, W. Bothe, G. Molière, W. Gentner, E. Bagge, and F. Bopp (Germany); L. Meitner, I. Waller, and H. Alfvén (Sweden); G. Occhialini, M. Cosyns, and C. Manneback (Belgium); L. Janossy (Ireland); C. Moeller (Denmark).

Most of the meetings took place in the famous Villa Olmo; in addition some evening sessions were organized in the Tempio Voltiano (Volta Shrine).

On September 11 the conference was officially opened by Enrico Fermi, who urged a closer cooperation among scientists of the world. The scientific meetings started on September 12. The morning session was devoted to the question of the origin of cosmic rays. E. Fermi (Chicago) presented a very clear picture of the present status of the problem, and he discussed some of the important ideas which have been proposed so far. He pointed out that his own theory, in which he proposes a new mechanism of accelerating charged particles in magnetic fields of our galaxy, gives a rather satisfactory explanation of cosmic ray observations made on primary protons. However, considerable difficulties arise in applying this mechanism to the heavy nuclei present in the cosmic radiation.

H. Alfvén (Stockholm), the discoverer of the magnetohydrodynamic mechanism of accelerating charged particles in free space, discussed the possibility that cosmic radiation may originate in the solar system. The main acceleration is supposed to take place in the sun's magnetic field. Solar activity, in particular magnetic storm effects, was also discussed.

E. Bagge (Hamburg) proposed that most of the cosmic radiation may originate beyond our own galactic system. His conclusions were mainly based on experiments of W. Rau, who claims to have found a variation of 0.7 percent in the intensity of the hard component with sidereal time.

J. Clay (Amsterdam) presented new data on the increase of the sea level intensity of cosmic rays during solar flares. In more than thirty instances, small variations of a few percent were noticed. Marcel Schein (Chicago) mentioned in the discussion that during a balloon flight on May 11 an increase of 50 percent occurred in the rate of nuclear disintegrations close to the top of our atmosphere (95,000 feet). This unusual increase must have been due to the large solar flare observed by A. H. Shapley (National Bureau of Standards) a few hours before the balloon flight took place (*Science*, 1949, **110**, pp. 159–161).

In the afternoon and evening sessions, problems concerning giant atmospheric showers and mesons were discussed. J. Clay introduced the subject of extensive showers by emphasizing the complexity of phenomena occurring in these very energetic events. He presented a number of new results on the absorption of secondary electrons and photons in air showers. E. Amaldi reported an experiment of the Rome group in which the frequency of high energy electrons occurring in extensive showers was studied. The results indicate that there are more electrons of very high energy present in these showers than expected from cascade theory alone. This result might, therefore, be considered as pointing in the direction of the assumption that extensive showers originate from nuclear collisions between very high energy particles.

J. Daudin (Paris) showed a number of interesting cloud chamber pictures of penetrating showers and discussed the relation between penetrating showers and air showers. R. Maze (Paris) reported on his counter studies of extensive showers.

C. Franzinetti (Bristol) and S. Rosenblum (Paris) presented the results of their experiments on the magnetic deflection of charged particles studied by the method of placing photographic plates between the pole faces of a large electromagnet. The magnet is located on Jungfraujoch at an altitude of 12,000 feet. According to Franzinetti and Rosenblum, it was not possible definitely to identify any new electrically charged elementary particles in addition to those already known to exist (electrons, μ-mesons, π-mesons, and protons). In particular there does not seem to be clear-cut evidence for the existence of the τ-meson of a mass around 1,000 electron masses, on the basis of these investigations. Further experiments are needed to settle this important question.

Another group of papers presented by the Bristol and Brussels groups dealt with the problem of the spontaneous decay and nuclear capture of μ-mesons. A. L. Hodson and A. Loria (Manchester) presented a paper in which they described a new method of controlling a Wilson cloud chamber by an internal counter. The interesting feature of this new method is that one can see the cloud chamber tracks of minimum ionization particles inside the counter. The counter itself consists of an inside wire of tungsten surrounded by six rods placed at the corners of a hexagon.

The meeting on September 13 was opened by C. F. Powell, who gave a detailed account of the work carried out under his direction in Bristol on transmutations produced by cosmic ray particles of great energy. A number of interesting new events of nuclear disruptions, found in electron-sensitive emulsions, were discussed. Some of these events are of particular importance, since they represent phenomena in which several mesons and nucleons are emitted from a single parent nucleus hit by a very high energy particle. A very spectacular nuclear disintegration (star with more than 50 prongs) of this type was described by L. Leprince-Ringuet (Paris) who reported on his investigations of high energy nuclear phenomena in cosmic radiation. The experiments were carried out partly in free balloons up to an altitude of about 96,000 feet. A picture of an interesting star of 52 prongs was shown, in which the collision of an energetic heavy nucleus of charge $Z \approx 17$ with a silver nucleus was clearly visible.

M. G. E. Cosyns (Brussels) gave an account of his investigations of stars containing showers of relativistic particles. A special analysis was made in order to establish the nature of the relativistic particles. By using the methods of grain counting and multiple scattering of tracks in photographic emulsions it was found that most of the particles carrying an ionization less than two times the minimum are mesons and those carrying an ionization between two and five times the minimum are protons. At mountain altitudes, most of these stars are generated by neutral primaries.

G. Occhialini (Brussels) gave a progress report on the techniques involving electron-sensitive nuclear plates. These plates have been available for a little more than a year. It is astounding what has already been accomplished in high energy physics through the efficient use of electron-sensitive emulsions. Occhialini discussed some new possibilities for the future, such as the magnetic deflection of tracks inside the emulsion. He pointed out that measurements on relativistic pairs seem to show that in this region of energy the grain density is not very sensitive to variations in the specific ionization.

G. Bernardini (Rome) presented interesting new data on cosmic ray stars having a smaller number of prongs (small stars). He discussed the nature of these stars and their origin in connection with the nucleonic component of the cosmic radiation.

M. Morand (Paris) gave a detailed description of a new method by which the east-west asymmetry is cosmic radiation was determined with the aid of tracks in nuclear emulsions. The results obtained so far indicate that we are dealing here with a promising method for future use in this field of cosmic ray research.

The reports on September 13 clearly demonstrated the usefulness of the photographic emulsion technique in the field of high energy physics.

The day of September 14 was devoted to an excursion to the University of Pavia, where a special celebration was held in honor of Volta's scientific achievements. The key address was delivered by L. Leprince-Ringuet, who was delegated by the French Academy to speak for M. de Broglie on the subject, "Deux grands savants d'il y a 150 ans: Laplace et Volta, tous deux membres de l'Institut de France."

The meeting on the 15th was opened with an address by P. M. S. Blackett (Manchester), who spoke on cloud chamber studies of penetrating showers at sea level. The measurements were carried out with a cloud chamber in a magnetic field of 7,000 gauss. A number of new results concerning the sign and charge of the penetrating particles, their momentum spectrum, and their angular distribution were presented. According to Blackett, the number of protons and mesons in penetrating showers is about equal at sea level. Some very interesting pictures were shown in which penetrating showers appeared to be produced by negative particles. Whether one is dealing with negative protons or negative mesons could not be decided from the evidence at hand.

R. B. Brode (Berkeley) reported on a number of interesting new investigations carried out in his laboratory. The mass spectrum of mesons was studied by means of accurately controlled cloud chambers on White Mountain (California) at an elevation of 12,000 feet. Some indication was found that singly charged particles of mass between 600 and 1000 electron masses (τ-mesons) may exist in the cosmic radiation. It seems remarkable, however, that all particles of this kind found so far carry a positive charge. By deflecting charged particles in magnetized iron an accurate measurement of the positive excess of mesons was carried out at sea level. Brode found a value of 1.32, which is somewhat higher than the value

previously reported by Bernardini and his collaborators. Brode mentioned that W. B. Fretter obtained some interesting cloud chamber pictures of penetrating showers originating in carbon.

The afternoon session was opened by a lecture of W. Heisenberg (Göttingen) on the problem of the creation of mesons in multiple processes. Heisenberg's ideas are based on the fundamental assumption that in a collision of a very high energy nucleon (proton and neutron) with another nucleon several mesons can be emitted. Heisenberg's theory leads to simple predictions regarding: (a) the frequency of meson showers (b) the angular distribution of emitted mesons, and (c) the energy spectrum of mesons. His theoretical predictions can be compared directly with the existing experimental material on large stars containing relativistic particles. A basically different point of view concerning the production of mesons was expressed by L. Janossy (Dublin) who presented the results of a joint paper with W. Heitler (Zurich). According to these authors, only one meson is produced in a single collision between two nucleons. That one actually observes several mesons emerging from the same nucleus must then be due to the fact that successive nucleon-nucleon collisions occur inside the same nucleus (plural production). Since meson production has not heretofore been studied on the hydrogen nucleus, Janossy pointed out that present-day evidence does not rule out the possibility that mesons of all energies are produced singly. On the contrary, Heitler and Janossy argue that the existing experimental data on the exponential absorption of the nucleonic component in our atmosphere can be deduced from their calculations based on the assumption of plural meson production.

G. Salvini (Milan) described a series of cloud chamber experiments on nuclear explosions which were carried out in the Italian High Altitude Laboratory at Testa Grigia (11,000 feet). According to Salvini, the ratio in the frequency of high energy nuclear events originating from lead to those from carbon was found to be about 2. This ratio can be deduced approximately from the theory of multiple as well as from the theory of plural production of mesons. Hence experiments of this kind do not enable us to decide which one of the two theories is the correct one.

O. Piccioni (Brookhaven National Laboratory) gave an account of his counter studies concerning the structure of penetrating showers. Piccioni used the method of delayed coincidences in carbon and sulfur to identify the π-mesons in penetrating showers. His experiments show that most of the particles traversing a few inches of lead are π-mesons. Piccioni found that the nuclear absorption in iron was considerably smaller for π-mesons than for nucleons.

G. D. Rochester (Manchester) reported some interesting observations on cosmic ray stars in photographic emulsions. The absorption of the star-producing radiation in air and in lead strongly indicates that most of the smaller stars in the lower atmosphere are produced by neutrons. A detailed analysis of stars shows that the proportion of α-particles is greater for small stars than for large ones, and it remains approximately constant for stars of more than six prongs. This is explained on the assumption that light nuclei are easily broken up by cosmic ray neutrons. Rochester made an energy analysis of a group of stars and he found that the majority of the charged particles below 30–40 Mev come from the evaporation of the atomic nucleus. The average nuclear temperature of a group of stars having from 7 to 17 prongs was found to be 5 Mev. A new important result was reported regarding the ratio of the number of recoil protons directly ejected from the nucleus to the number of particles evaporated from it. Rochester found that this ratio is of the order 1: 10.

D. H. Perkins (London) presented a number of interesting new results regarding nuclear explosions at high energies. In the small cosmic ray stars the energy distributions of α-particles and protons are consistent with a process of evaporation. In large stars there is a marked change in behavior between protons and α-particles; a mechanism different from evaporation has to be postulated for the emission of α-particles. It was found that the ratio of α-particles to protons increases with the prong number up to a maximum at nine prongs, and then decreases for larger star sizes. Perkins found that nuclei heavier than α-particles are ejected from stars in both ground and excited states. Particles of charges up to $Z = 10$ and energies up to 300 Mev have been observed. For example, among these nuclear fragments Be^6, Be^9, and C^{12} have been found. In contrast with α-particles in large stars, most of the heavier splinters have energies in excess of the Coulomb barrier potential.

On September 16 the first speaker was L. Janossy (Dublin) who discussed the question of the possible existence of a very lightweight meson (λ-meson) in extensive showers. Janossy pointed out that his recent experiments do not support his former conclusions regarding the presence of such particles in large air showers.

Marcel Schein (Chicago) presented a paper on the production of nucleons and mesons in cosmic radiation. He described a number of experiments carried out by the Chicago group in free balloons up to an altitude of 100,000 feet. It was found that the frequency of smaller nuclear explosions as a function of altitude follow an exponential increase up to the highest elevations reached. This result shows that at altitudes as high as 100,000 feet a large number of secondary neutrons and protons are present in the atmosphere. These secondaries must arise in part from the large nuclear disintegrations produced by the primary radiation and in part from the breaking-up effect of heavy nuclei in the highest layers of our atmosphere. Schein then discussed the problem of the production of low energy mesons studied in photographic emulsions. A number of events were shown in which a collimated beam of relativistic particles was visible. It was pointed out that events in which a very narrow bundle of such particles occur represent some evidence for the multiple production of mesons. The quantitative data obtained on the prong distribution of minimum ionization particles and on the zenith angle effect of the large stars could not be compared with the theories of

Heisenberg and Heitler-Janossy, as these theories were not known to the author before the meetings at Como.

E. P. George (London) spoke on nuclear interactions of cosmic ray particles. A number of interesting experiments on penetrating showers were carried out at sea level and on Jungfraujoch. Transition curves of penetrating showers in Pb, Al, and paraffin approach the same limiting value at large absorber thicknesses. According to George, this fact supports the concept of multiple rather than plural meson production. George studied nuclear explosions in photographic emulsions at a depth of 60-meter water equivalent below the surface of the ground. A small number of stars were found at this depth. It was proposed that there may exist a small interaction between μ-mesons and nuclei, which may account for the presence of stars underground.

J. G. Wilson (Manchester) described a series of very accurate experiments carried out on the positive excess of mesons. He used a specially constructed magnetic spectrograph which enabled him to determine the positive excess as a function of meson energy. Wilson found that the positive excess reaches a maximum of 1.32 at meson energies around two Bev. The drop toward higher energies is very slow and somewhat faster on the low energy side. Wilson's results on the positive excess are of importance for our understanding of the mechanism of meson production in the energy range of several Bev.

This summary represents only a selection of the many interesting papers which were given at the meetings. The writer wishes to apologize for the fact that not all of the papers could be included in this present report. The great majority of the lectures were given in English.

The scientific sessions closed at noon with an enthusiastic speech by Bernardini (Rome), who summarized the achievements of the conference. The afternoon was devoted to an excursion on beautiful Lake Como to the famous resort town of Bellaggio, where a splendid farewell dinner was offered, embellished by speeches from our hosts and from some of the leading participants of the meetings, expressing gratitude and appreciation in the name of all who had been present.

After the official closing of the conference more than a hundred of the scientists joined in a second excursion, this time going to the Italian High Altitude Laboratory at Testa Grigia (11,000 feet) which is beautifully located on the west side of the Matterhorn. Everyone was impressed with the great amount of work going on there.

We all should be very thankful to those men who expended so much effort to make the Como Conference a memorable occasion.

Report on International Symposium on the Biology of High Altitudes

David Bruce Dill, ***School of Aviation Medicine, Randolph Field Air Base, San Antonio, Texas***

Under sponsorship of Unesco and the government of Peru, an international symposium was held in Lima, Peru, November 23–30. The organizing committee, appointed by the Peruvian government, was headed by Carlos Monge M., director of the National Institute of Andean Biology. Bernardo A. Houssay of Argentina was honorary president of the symposium.

Delegations represented most of the countries of North and South America as well as Switzerland, France, and Italy. The U. S. sent an official delegation of six, headed by Harry G. Armstrong, Surgeon General of the U. S. Air Force. Eighteen other scientists represented the Air Force, the Navy, the Army Chemical Corps, the Research and Development Board, and the Aero-Medical Association. This party included a member of the British Joint Services Mission. Other U. S. agencies represented included the National Institutes of Health, the U. S. Department of Interior, the Carnegie Institution, and the Institute of Inter-American Affairs.

The program centered around man at rest and at work in the environments characteristic of different altitudes. The subjects included adaptation and acclimatization to altitude in man and animals; the acute and the chronic effects of altitude in rest and work; the relations between fertility and altitude; and the special problems of aviation medicine.

About half of the scientific program was devoted to 15-minute papers, with plenty of time for discussion. In other sessions, experts led round table discussions of selected general topics. Some papers were presented in English. A translated summary of Spanish papers was frequently provided. These, with brilliant off-the-cuff translating by Albert Hurtado of the Andean Institute and E. S. G. Barron of Chicago, lowered the language bar to interchange of ideas.

Dr. Monge philosophized on the Incan organization for protecting their people against what he termed climatic aggression. Physical training was made a routine part of living; wedding ceremonials favored survival of the fittest; the colonization system recognized the phenomenon of acclimatization. Dr. Monge also described chronic mountain sickness, first recognized by him in 1928 and now known as Monge's disease. It illustrates failure to resist climatic aggression and its specific cure is residence at sea level.

Dr. Hurtado led a stimulating discussion of chronic anoxia as seen in the Indian miner at Morococha, 14,900 feet. His acclimatization depends on ancestry, on uninterrupted hypoxia from the beginning of fetal life, and on hard physical work. In his acclimatized state, he exhibits hyperventilation, appreciable in rest and marked in exercise; increased alveolar volume; normal pH; polycythemia; normal resting cardiac output; and greater vascularity of tissues. At that altitude the Indian miner has as great a work capacity on the treadmill as the Indian at sea level, and he is more efficient. This new

finding has raised unsolved questions about the possibility of fundamental changes in the biochemical processes of energy transformation.

In a study participated in by Dr. Rotta, it was found that the Morococha miner has an absolute as well as a relative increase in blood hemoglobin. He has three-eighths more red cells per unit volume of blood and seven-eighths more red cells in circulation. His blood volume is two liters greater; the increase is entirely red cells. In terms of hemoglobin he has about 1,450 grams, as compared with about 800 grams in his sea-level cousin.

The dynamic state of man is well illustrated by his hemoglobin response. A new balance must be struck between rate of formation and rate of destruction; this balance must also adjust to the increase in the capillary volume. This process and the numerous other steps in acclimatization take months, possibly years, for attainment of equilibrium. Sometimes there is failure of some element. Then physiology retreats before pathology.

Through the hospitality of the institute, the delegates were privileged to visit the remarkable high altitude laboratories at Huancayo (3,200 meters), Morococha (4,540 meters), and Ticlio (5,030 meters). The party witnessed the dedication of the new building of the Morococho laboratory. These laboratories have been made possible by the government of Peru, the Rockefeller Foundation, and Don Manuel Peilago of Huancayo. Staff members expressed the hope that scientitsts interested in any phase of high altitude biology will make use of the laboratories.

All of the delegates were impressed by the high quality and wide range of the institute's research program, the excellence of laboratory facilities at Lima and at altitude, and the generous hospitality of the people of Peru. The Institute of Andean Biology will undoubtedly become as famous internationally as the Naples Biological Station, the Jungfraujoch, and the Woods Hole Biological Laboratory.

About People

Erwin E. Nelson, adjunct professor of pharmacology at the George Washington University School of Medicine, has been appointed medical director of the Food and Drug Administration of the Federal Security Agency. Dr. Nelson assumes his new duties this month when the resignation of **Robert T. Stormont** becomes effective.

Griffith Conrad Evans, professor of mathematics, has been named Faculty Research Lecturer on the Berkeley campus of the University of California for 1949–50. Dr. Evans, who is an authority on potential theory, will deliver his lecture in March.

Guido Castelnuovo, professor emeritus, Department of Mathematics, University of Rome, and president of Accademia Nazionale dei Lincei, has been made Senator in recognition of his achievements in mathematics.

Walter M. Scott and **John R. Matchett** have been appointed assistant chiefs of the U. S. Department of Agriculture's Bureau of Agricultural and Industrial Chemistry. **Charles H. Fisher,** head of the Carbohydrate Division of the bureau's Eastern Regional Research Laboratory, at Wyndmoor, Pennsylvania, will succeed Dr. Scott as director of the Southern Regional Research Laboratory in New Orleans.

Dr. Matchett was formerly coordinator of food research at the bureau's Western Regional Research Laboratory in Albany, California.

Neil M. Judd, a member of the U. S. National Museum anthropological staff, since 1911, and curator of archaeology since 1919, retired on December 31 to devote his full time to writing. Dr. Judd was named honorary associate in anthropology at the museum on January 1.

William James Price, former research engineer with the Battelle Memorial Institute, and **David W. Lueck,** research associate at the University of Washington, have been appointed staff members of the College of Engineering Sciences, U. S. Air Force Institute of Technology, Wright-Patterson Air Force Base, Dayton, Ohio.

Herta Meyer, of the Research Association of the Institute of Biophysics, University of Rio de Janeiro, has been a visitor the past two months in the Tissue Culture Laboratory, University of Texas. Miss Meyer has developed important new research techniques for the study of intracellular parasitism which are being adapted by the laboratory to methods of tissue culture. Miss Meyer will visit medical centers here and in England before returning to South America.

Grants and Awards

The American Institute of Electrical Engineers will present its 1949 **Edison Medal** to Karl B. McEachron, of the General Electric Company, at its January 30–February 3 meeting in New York City. Dr. McEachron will receive the medal for his contribution to the advancement of electrical science in the field of lightning and other high voltage phenomena and for the application of this knowledge to the design and protection of electric apparatus and systems.

Wendell P. Woodring, geologist, U. S. Geological Survey, and an authority on the paleontology of the West Indies, has been awarded the **Penrose Medal of the Geological Society of America.** Dr. Woodring was honored for his researches in the West Indies and Central and South America. The medal, which is the society's highest award, is bestowed only in those years when an outstanding candidate for the honor is presented.

The $1,000 prize of the American Association for the Advancement of Science, given each year for a noteworthy paper presented at the AAAS annual meeting, was awarded at the close of last week's New York meeting to Armin C. Braun, associate member of the Rockefeller Institute for Medical Research. Dr. Braun's paper described his experiments on the development and poliferation of plant cancers caused by crown-gall bacteria—studies which the prize committee called "models of simplicity—and so well designed that they chart a basic course in man's understanding of the greatest human malady."

Two factors influencing crown-gall development were found: one changing normal cells to cancer cells, the other (probably a hormone) making it possible for the transformed cells to multiply. The first factor was stopped by exposing the plant to a temperature of 30 degrees Centigrade or more but the second factor was not, so that it was possible to study the two processes separately.

The studies also showed that a wound in the plant was required to introduce the bacteria and that a time factor was involved in the cancer development. "Very little, if any, transformation occurs during the first twenty-four hours after inoculation," Dr. Braun stated. "It is during the subsequent ten and more hours that measurable changes take place. As a result of this work it is possible to show that a definable threshold concentration of tumor-inducing principle is necessary to transform normal plant cells to tumor cells.

"Further experiments revealed that the host cells became immune from carcinogenic influence of the bacteria four days after inoculation. This occurred despite the fact that many virulent bacteria were in intimate contact with the host cells after the fourth day. During the four-day period the plants were grown at a temperature inhibiting the cancer-producing principles.

"It appears, therefore, that it is before and during early stages of active wound healing that normal cells are converted into tumor cells."

Dr. Braun also observed that growth of the transformed cells was independent of the inciting bacteria and that bacteria-free tumor cells maintained in culture for more than five years "retained undiminished their capacity for rapid, unrestrained, and uncoordinated growth."

Georg von Bekesy of the Harvard Psycho-Acoustic Laboratory has been named the first recipient of the **Shambaugh Prize** recently established by the family of the late George E. Shambaugh, noted otologist. The prize is awarded biennially for outstanding research in hearing. The recipient is chosen by the Collegium Oto-Rhino-Laryngologicum, Amicitiae Sacrum.

The Theobald Smith Award in Medical Sciences for 1949 was presented to Seymour S. Kety, professor of clinical physiology, Graduate School of Medicine, University of Pennsylvania, at the annual meeting of the AAAS, held in New York City last week. The award, consisting of $1,000 and a bronze medal, was established in 1936 by Eli Lilly and Company and is given for "demonstrated research in the field of the medical sciences, taking into consideration independence of thought and originality." It has been five years, owing to the war, since the award has been granted. Dr. Kety was cited primarily for his investigative work on "The Quantitative Measurement of Blood Flow and Oxygen Consumption of the Human Brain." He has been author or coauthor of 44 publications dealing with this research problem.

Past recipients of the award are Robley D. Evans, professor of physics, Massachusetts Institute of Technology, Cambridge; Charles F. Code, Department of Astronomy and Astrophysics, Yerkes Observatory, Williams Bay, Wisconsin; Albert B. Sabin, professor of pediatrics, Children's Hospital Research Foundation, Cincinnati, Ohio; Herald R. Cox, director of virus research, Lederle Laboratories, Inc., Pearl River, New York; and Sidney C. Madden, professor and head of the Department of Pathology, Emory University School of Medicine, Atlanta, Georgia.

Fellowships

The Conway MacMillan Memorial Research Fellowship in Botany is being offered at the University of Minnesota to doctoral students for the year 1950–51. This $1,200 award was established in 1946 by the late Charles J. Brand, of Washington, D. C. According to the terms of the gift, special mention is made of students from the University of Chile and the Catholic University of Chile. Applications should be made by *March 1.* Inquiries should be addressed to A. O. Dahl, Chairman, Conway MacMillan Fellowship Committee, Department of Botany, University of Minnesota, Minneapolis 14.

The following postdoctoral fellowships and scholarships are available at **Ohio State University** for 1950–51: *University Postdoctoral Fellowship,* $3,000, October 1, 1950–June 30, 1951, for research work in any of a variety of fields of academic interest. *Elizabeth Clay Howald Scholarship,* $3,000, July 1, 1950–June 30, 1951, for research in any of a number of fields. Several *Mary S. Muellhaupt scholarships,* July 1, 1950–June 30, 1951, with stipends ranging from $1,800 to $3,000, for research work in bacteriology, botany and plant pathology, physiology, and zoology and entomology.

Application blanks may be obtained from the Dean of the Graduate School, Ohio State University, Columbus, Ohio. Applications must be filed by *April 1.*

Industrial Laboratories

Charles E. Dutchess, medical director of Schenley Laboratories, Inc., has been elected president of the Association of Medical Directors, which represents the principal pharmaceutical firms in the nation. Dr. Dutchess was vice president of the association during the past year.

Zay Jeffries, vice president of the General Electric Company in charge of the Chemical Department, retired from the company December 31. Dr. Jeffries has been a member of the company's four-man committee

which administers the Nucleonics Project.

Richard H. Zinszer, formerly director of research for the Union Oil Company at Wilmington, California, has accepted the position of technical director of the **Arabian-American Oil Company,** New York City.

Meetings and Elections

A symposium on recent advances in cardiovascular surgery will be held January 21 at the Mayflower Hotel, Washington, D. C., under the sponsorship of the Surgery Study Section, National Institutes of Health, Bethesda, Maryland. Frederick A. Coller, University of Michigan, is chairman of the section, and Claude S. Beck, of Western Reserve University, is chairman of the Arrangement Committee. Further information can be obtained from J. Marshall Ellis, Executive Secretary, Surgery Study Section, National Institutes of Health.

The Tennessee Academy of Science at its recent meeting elected the following officers for the ensuing year: president, Samuel L. Meyer, Department of Botany, University of Tennessee; vice president, M. L. McQueen, Mathematics Department, Southwestern College; secretary, Arlo I. Smith, Biology Department, Southwestern College; treasurer, James J. Friauf, Biology Department, Vanderbilt University.

The Institute of Medicine of Chicago elected the following officers for 1950 at its annual meeting this month: Henry T. Ricketts, chairman of the board; Stanley Gibson, president; Otto F. Kampmeier, vice president; George H. Coleman, secretary; and E. Lee Strohl, treasurer.

The American Standards Association elected three new members to its board of directors, to take office January 1 for a three-year term: Maurice Stanley, chairman of the board of the Fafnir Bearing Company, represents the Antifriction Bearing Manufacturers Association, Inc.; J. G. Vincent, vice president, Packard Motor Car Company, represents the Automobile Manufacturers Association; and B. S. Voorhees, vice president, New York Central System, represents the Association of American Railroads.

Miscellaneous

The National Bureau of Standards is now making available high purity standard samples of organic sulfur compounds to industrial laboratories. The compounds are prepared under the sponsorship of the American Petroleum Institute, by the Bureau of Mines, U. S. Department of the Interior, where they are used for the measurement of needed physical, thermodynamic, and spectral properties. Instructions for ordering may be obtained from the National Bureau of Standards, Washington 25, D. C. Directions for transferring the samples in vacuum are also available from the bureau on request.

Invertebrate zoologists and students of biogeography will be interested in recent developments in the reconstitution of the **Zoologisches Museum of Hamburg,** as reported by G. E. Hutchinson, of the Yale Zoology Department, who visited the museum during his recent visit abroad.

The Zoologisches Museum housed the greatest collection of Oligochaets in the world, amassed, among other important material, by the late Wilhelm Michaelsen. In view of the importance of the earthworm in the study of zoogeography and evolution, as well as its economic and ecological importance, Dr. Hutchinson considers the fate of this collection of unusual scientific interest.

"The Michaelsen collection," stated Dr. Hutchinson, "along with other very important collections in liquid preservative, numbering in all about a quarter of a million jars, was stored during the war in an abandoned subway. Some postwar loss of alcohol was reported on account of attempts by blackmarket liquor dealers to steal the preservative from a part of the collection. The loss, however, was not serious. But is was feared that ordinary evaporation would lead to complete deterioration of the collections if they remained inaccessible. Since the museum building was entirely gutted as the result of aerial attacks during the war and the greater part of the library destroyed, there appeared no immediate possibility of rescuing the collection. During the summer the British authorities at Hamburg became interested in the matter and Professor B. Klatt, the distinguished director of the museum, now writes that he has been assigned a disused bunker or concrete air raid shelter to which the collections are being transferred. This will make possible the unpacking and proper care of the material. At a time when every available structure in the city is in demand, this far-sighted action on the part of the British Commissioner, John K. Dunlop, will be appreciated by zoologists throughout the world."

Recently Received—

Engineering College Research Council American Society for Engineering Education Review of Current Research and Directory of Members Institutions, 1949. College of Engineering, State University of Iowa, Iowa City, Iowa.

Virginia Academy of Science 1948–49 Proceedings. Richmond, Va.

Isotopes: A Three-Year Summary of U. S. Distribution. U. S. Atomic Energy Commission. U. S. GPO, Washington 25, D. C. 45 cents.

Investigation Into the U. S. Atomic Energy Project: Appendixes to Hearing Before Joint Committee on Atomic Energy. 81st Congress. First Session, Part 23. U. S. GPO, Washington 25, D. C.

Calories Make A Difference: Report of Studies on Three Groups of Children. Pauline Beery Mack. Sugar Research Foundation, Inc., 52 Wall Street, New York City.

Index to the Literature of Food Investigation, Department of Scientific and Industrial Research, Great Britain. Compiled by Agnes Elisabeth Glennie. His Majesty's Stationery Office, London, England. 6 s.

Council for Scientific and Industrial Research, 22nd Annual Report for Year Ended June, 1948. Parliament of the Commonwealth of Australia. L. F. Johnston, Commonwealth Government Printer, Canberra, Australia.

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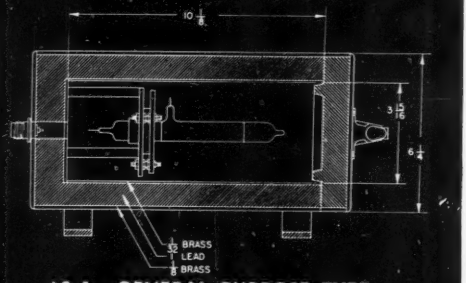

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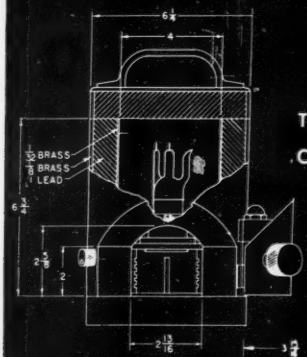

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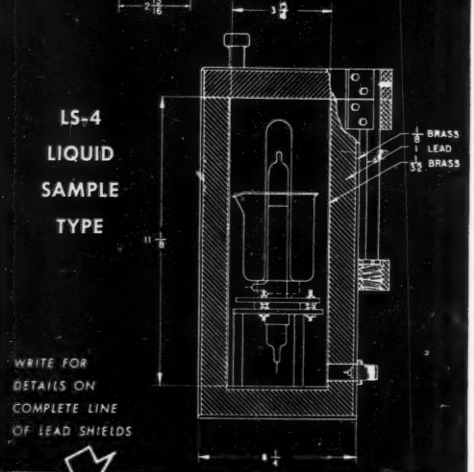

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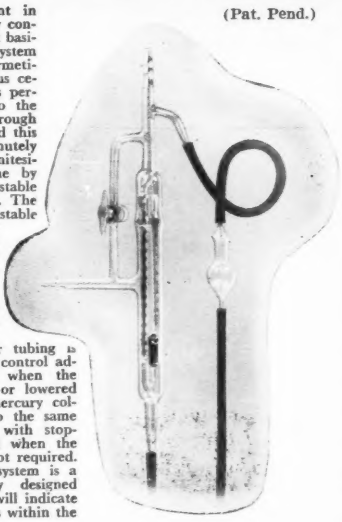

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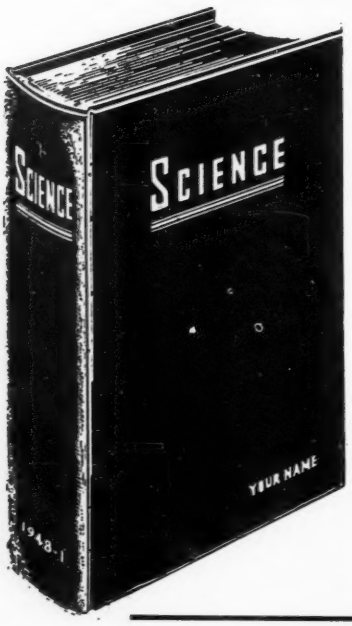

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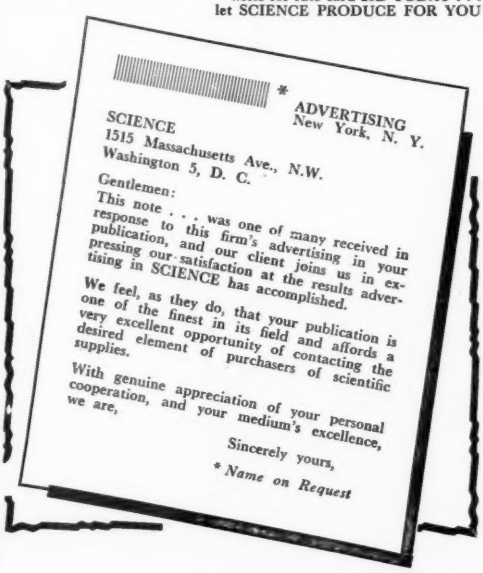
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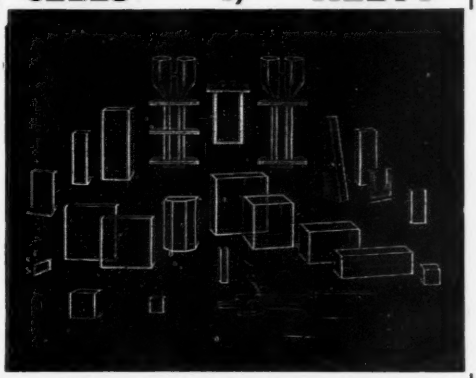

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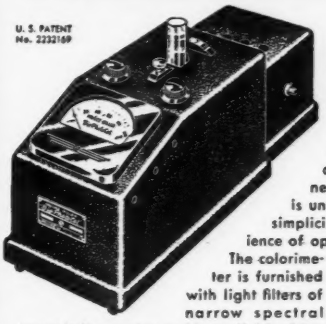

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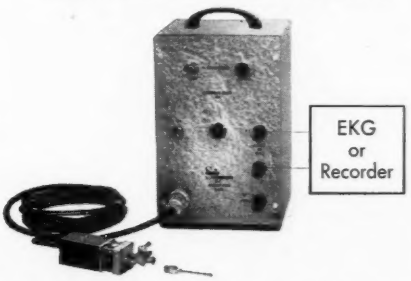

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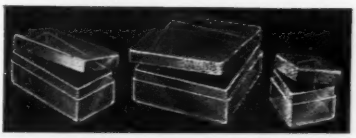

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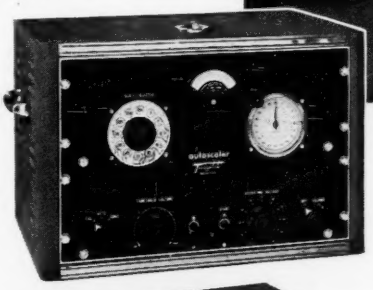

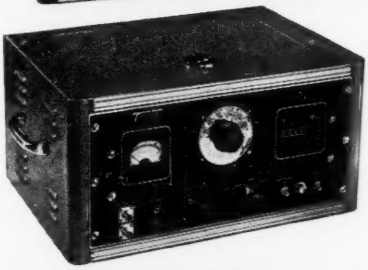

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The new "100" Scaler has all the features of the "64" Scaler. In addition, all readings are given directly in a decimal scale and require no interpolation. This instrument uses not only two replaceable Decascales, but also has a plug-in type Discriminator Input Circuit for simplified maintenance.

Write for Bulletins describing these and other instruments designed especially for the radioactivity laboratory.

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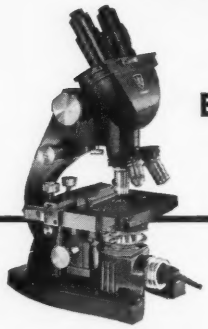

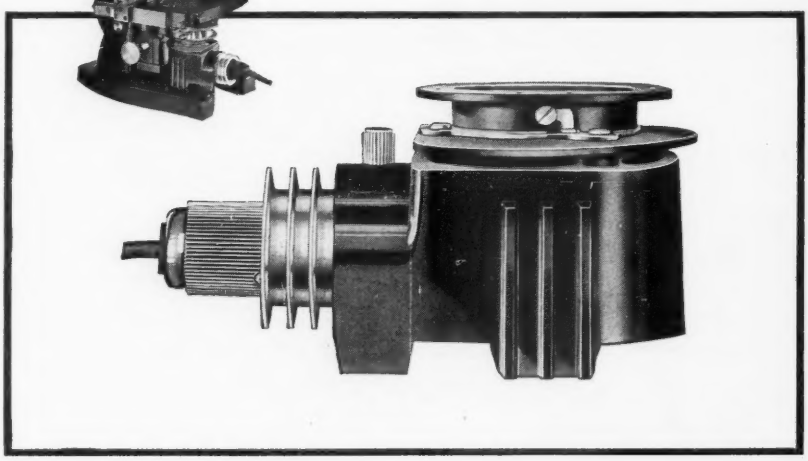

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